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SOME FUNGUS PARASITES OF ALGAE[1]

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(WITH EIGHT FIGURES)

About twelve years ago I was giving considerable attention to the study of the parasites of the algae in the vicinity of Ithaca, N. Y. At that time I hoped that the investigations might eventuate in a monograph of the Chytridiales of the Cayuga Lake basin. The pressure of other investigations has almost completely interrupted these studies. Because of our limited knowledge of the occurrence and habits of these interesting fungi in North America, it has seemed to me desirable that the observations already made should be recorded, in the hope that this may stimulate a greater interest in these plants.

As a result of the studies three papers have already been published. An extended paper on the genus Harpochytrium in the United States was published in 1903,[2] a summary of which later appeared in the *Journal of mycology* in 1904.[3] A short note on the interesting behavior of the zoospores of *Rhizophidium globosum* while escaping from the zoosporangium was published in 1894.[4] This behavior related to the habit of their sensing or feeling the exit opening in the sporangium, which they do by means of pseudopod-like extensions of the protoplasm in different directions, after having come to rest on the inside of the zoosporangial wall. In case they happen to come to rest close by the exit they "feel" it by one of the pseudopods, and slide out.

[1] Contribution from the Department of Botany, Cornell University, No. 133.

[2] ATKINSON, G. F., The genus Harpochytrium in the United States. Ann. Myc. **1**:479–502. *pl. 10.* 1903.

[3] ———, Note on the genus Harpochytrium. Jour. Myc. **10**:3–8. *pl. 72.* 1904.

[4] ———, Intelligence manifested by the swarm-spores of *Rhizophidium globosum*. BOT. GAZETTE **19**:503, 504. 1894.

In case they are distant from the exit, not finding it they round up into the motile zoospore form again and swarm around in the zoosporangium for a time, and coming to rest make another trial. It is evident that when the zoosporangium is filled with the zoospores the latter will escape quite rapidly for a time because so many of them are crowded successively against the exit. As the numbers diminish there is greater freedom for swarming. The zoospores then swarm around and around in great circles inside the wall of the zoosporangium, now and then coming to rest and feeling around for the exit. This same behavior has been observed in the case of a number of other species. In addition to this a quite remarkable phenomenon was observed in the case of another species, *Rhizophidium brevipes*, which will be described below.

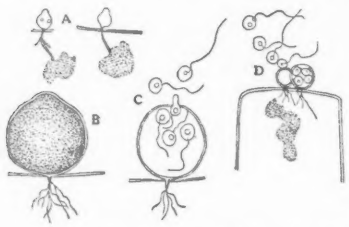

FIG. 1.—*Rhizophidium globosum* (A.Br.) Schröt. *A* young plant, shortly after germination of zoospores with germ tubes, penetration tube forming rhizoids; *B* mature plant ready to form zoospores; *C* zoospores escaping; *D* much smaller plants.

In the presentation of these observations I shall make no attempt to arrange the genera in any natural order of relationship, this matter being reserved for a future work. Since a number of species of Rhizophidium were studied I will begin with this genus.

RHIZOPHIDIUM BREVIPES

This was collected in a pool beyond Forest Home, N. Y., a little more than one mile from Ithaca. It was attached to the wall of a fruiting cell of *Spirogyra varians*. The zoosporangium is oval with a small apical papilla. The wall shows two distinct layers, an outer rather thick one and an inner thin one. At the time of the maturity of the zoospores the papilla of the outer layer becomes gelatinized at the apex, forming a minute opening about 4 μ in diameter.

One very characteristic feature of this species is the very rudimentary condition of the rhizoids. The very slender branched rhizoids so characteristic of *R. globosum* and other species appear to

be absent. The penetration tube of the zoospore forms a short stalk, which projects but a short distance within the cavity of the host cell. This rudimentary condition of the rhizoids recalls that of *Harpochytrium hedenii* Wille, though in the latter it penetrates only the outer lamella of the wall and flattens out in the form of a disk in the middle lamella, or merely penetrates a thin layer of slime on the host and flattens out on the outer wall (ATKINSON 1903). This penetration tube serves also as the absorbent organ for such food as the plant obtains from the fluids within the gametangium of the host surrounding its zygospore. The zoosporangium measures 21–24 μ, while the zoospores are about 3 μ in diameter, with a single cilium and a prominent oil drop.

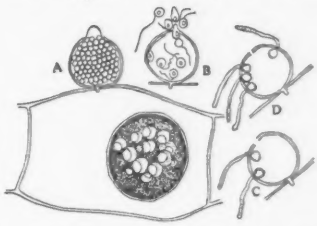

FIG. 2.—*Rhizophidium brevipes* Atk. *A* mature zoosporangium crowded with zoospores, outer layer of wall at exit pore dissolving, inner layer projecting as a papilla before the rupture, plants attached to a gametangium of Spirogyra containing zygospores; *B* zoospore escaping; *C* two zoospores, unable to escape by exit pore, have germinated and are attempting to escape through germ tube, protoplasm in the apex of the tube; *D* same later, showing how the zoospores retreated from the first tubes and after swarming around for the second time attempted to escape again by the germ tubes.

The species was first observed in a cell preparation which was made on April 24, 1895, at about 4 P. M. The zoosporangium was mature and the protoplasm finely granular. In the course of half an hour from the time of the first observation, the granules began to arrange themselves in numerous small groups, the beginning of the formation of the zoospores. Very soon these began to disappear and were gradually replaced by a prominent oil drop for each group of granules. Each oil drop was surrounded by a hyaline mass of homogeneous protoplasm. The preparation was watched for the greater part of the time from 4 P. M. until 6 P. M., and fresh water was frequently drawn in, in order to hasten the development and maturity of the zoospores. At this time it appeared that the zoospores would not become mature under an hour, and the preparation was placed in a moist chamber until 7 P. M. It was then examined and fresh water added. The apical portion of the outer wall was dissolved;

soon the inner wall at this point became ruptured, and precisely at 7 P. M. the zoospores began their escape. The number of zoospores was very large and they were packed so tightly in the zoosporangium that it was impossible for them to make other than slight amoeboid movements. Those at the apex slowly moved through the opening one or two at a time, so that the number could be readily counted. In passing through the opening the zoospore puts forth a stout hyaline projection which feels and leads the way, and then the body slowly moves through. On the outside, at the mouth of the zoosporangium, each zoospore rests for a short time, during which a few plastic movements are exhibited; then it rounds off and darts away.

When 50 or 60 zoospores had made their escape in this way the space in the zoosporangium was not so crowded, and a few of those at the center began active swarming movements, while those at the periphery of the sporangium were quiet or only exhibited plastic movements, and those nearest the opening continued to escape in the manner described. This continued until there were very few still within the zoosporangium. The remainder of the zoospores were all swarming, and at times some of them would come to rest on the side of the wall, put out by amoeboid movement a short pseudopodium, and feel evidently for the opening. Not finding it they would swarm around within the zoosporangium again, and again come to rest, maneuver for the opening, and on finding it escape. This manner of finding the opening is the same as that described for *R. globosum* (ATKINSON 1894), but as the opening is larger than in that species, the body of the zoospore does not become constricted at the passage. At 7:18 P. M. all but four of the zoospores had made their escape, and in four minutes more two of these had escaped, leaving two still within the zoosporangium. These were watched for an hour longer, and they divided the time in swarming and feeling for the exit, but were unable to escape; though several times they located themselves directly at the opening, they seemed to be insensible of it.

The preparation was placed in the moist chamber and left for the night. On the following morning both of the zoospores were found located on one side of the zoosporangium and each had germinated.

The slender germ tube, having penetrated the wall of the zoosporangium, extended in a tortuous course for a distance of 15–20 μ. The preparation was examined several times during the day. Since one of the zoospores was located not far from the wall of the spirogyra, it was hoped that the thread would find the cell and enter it, inasmuch as it was pointed in that direction. The preparation was left in this condition during another night, the slender tube of the zoospore nearest the spirogyra cell not yet having reached it. On the following morning the preparation was examined again, and I was surprised to find that one of the zoospores was on the other side of the zoosporangium. Close examination showed that both had moved during the night. Not being able to find any suitable nourishment, the protoplasm in each germinating cell had migrated back from the inclosing membrane into the zoosporangium, formed a zoospore, and had passed through a second swarming period. Then coming to rest again they had germinated, the germ tube of each having again penetrated the wall of the sporangium and formed a slender tube 15–20 μ long. The preparation was kept for a day longer, but the zoospores did not form again.

This behavior of the zoospores is quite interesting, since it manifests not only a sort of "ingenuity" in the attempt to escape from the zoosporangium, but, what is more important, the tendency, under certain conditions of existence which prevent the zoospore from seeking a host cell by the normal planetic method, to migrate in the form of a true mycelial tube, which is quite different from the very delicate slender absorbent rhizoids normally developed from the germ tube in other species of the genus.

Rhizophidium brevipes, n. sp.—Zoosporangia oval, 21–24 μ in diameter, with a papilla at the apex, in which the exit pore is formed. Rhizoidal apparatus very rudimentary, consisting of the short, blunt, unbranched entrance tube of the zoospore, which projects but a very short distance beyond the inner lamella of the wall of the host. Zoospores oval with an oil drop, uniciliate, 3 μ in diameter.

On walls of gametangia of *Spirogyra varians* in which zygospores are formed. Vicinity of Ithaca, N. Y.

Zoosporangiis ovatis, apic[illegible]uno, brevi, papilliformi ostiolo, basi, uno brevi, non ramoso tubulo. Zoosporis ovoidiis, 3 μ latis, una guttula hyalina et uno cilio praeditis.

RHIZOPHIDIUM SPHAEROCARPUM

Rhizidium sphaerocarpum Zopf, Nova Acta Leop.-Carol. Deutsch. Akad. **47**:202. *pl. 19. figs. 16–27.* 1884.

Rhizophidium sphaerocarpum A. Fischer, Rabenh. Krypt. Flora Deutschl. Oesterr. u. Schweiz 2 Aufl. **4**:95. 1892.

What I have taken to be this species occurred in abundance on threads of *Mougeotia parvula* collected in a gutter on Thompson St., Ithaca, N. Y., April 7, 1895. The zoosporangia are oval in form and vary considerably in size, the larger ones measuring 16–18× 18–20 μ, while the smaller ones are about 10×11 μ. They occur singly or in groups of two to four, the smaller ones more commonly in groups and the larger ones rarely so. The rhizoids are very much reduced, consisting of a few very short branches from the short entrance tube.

The wall of the zoosporangium consists of two lamellae, an outer stout lamella and a thin inner membrane. This is very well seen in the dehiscence of the zoosporangia. At maturity the apex of the outer wall of the zoosporangium dissolves, forming a large circular opening for the exit of the zoospores. Through this the inner membrane projects in the form of a short broad papilla by the swelling of the epiplasm. The final rupture of this membrane sets the zoospores free, and the large opening permits their rapid escape. The exit pore measures 5–6 μ in diameter in the larger forms, and in the smaller ones is proportionately larger, being 4–6 μ in diameter, some of the smaller zoosporangia appearing like cups when open. The zoospores are oval, possess a long cilium and a prominent oil globule, and measure 1.5–2 μ in diameter. In germination, if the germ tube is directed toward the wall of the host cell, it penetrates the wall, and then the zoospore enlarges to the size of the mature plant, becoming the zoosporangium. If, however, as sometimes happens, the germ tube is directed away from the wall, or along its surface, it may grow to a considerable distance, sometimes reaching a length of 30 μ. The zoosporangia sometimes grow so as to appear attached by their side instead of by their base, and the opening is then at the side (*fig. 3, C*).

In nearly all of the larger specimens (which may prove to be a different species) the effect on the host cell was quite remarkable. The host cell not only becomes considerably larger near the middle,

but is very much elongated. Frequently at the middle it is not much larger, while on each side an enlargement occurs (*fig. 3, D, G*). The stimulus which causes the increase in the length and size of the cell also increases the development of the chromatophore. When this influence ceases the chromatophore begins to degenerate, the color

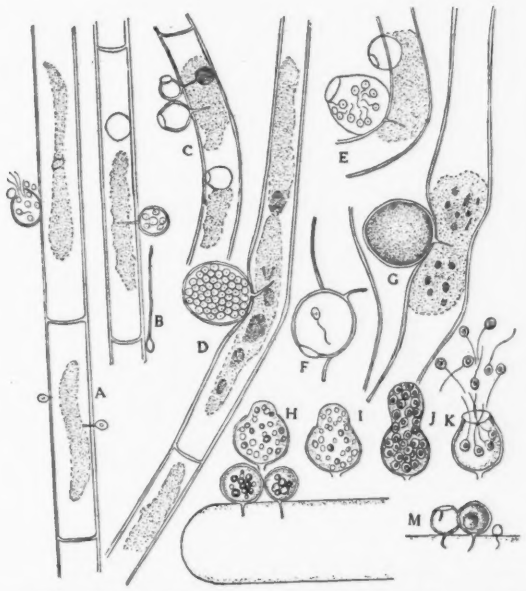

FIG. 3.—*Rhizophidium sphaerocarpum* (Zopf) Fisch. *A* filaments of Mougeotia showing entrance of germinating zoospores, and mature plant with zoopores escaping; *B* zoospore germinating, with germ tube growing parallel with the host thread, mature plants also attached to the host; *C* mature empty zoosporangia, showing one with the penetration tube on the side; *D* mature plant, large form, showing hypertrophy and curving of host cell; *E* mature plant with escaping zoospores, showing strongly curved host cell; *F, G* showing hypertrophy of host cell; *H–M* different stages in the opening of the zoosporangium and escape of the zoospores; *A–G* original; *H–M* after ZOPF.

fades, the structure breaks down, and the processes of dissolution separate it into small particles with the pyrenoids more distinct, all coalescing into an amorphous mass, which gradually flows toward the middle of the cell where the sporangium is located. This may be due partly to the fact that the cell of the host is larger at that point, as well

as to an influence which the parasite exerts upon it, for it is possible that the crowding of the chromatophores from each side toward the middle may cause the two enlargements near the middle with the constriction between them (*fig. 3, G*). Not only does the influence of the parasite excite these changes in the host cell, but it also causes the cell to become more or less strongly arched at the point of insertion of the sporangium, the sporangium being in the concavity of the arched thread. This effect has only been noted in connection with the larger forms, except where the smaller ones are several in a group, and here the curving of the thread is slight in comparison with that which exists in the case of the larger sporangia. ZOPF (*l. c.*) does not mention any similar hypertrophy of the host cell caused by this species, nor is it shown in his illustrations. He figures the plant on Spirogyra. A. FISCHER (*l. c.*) reports it on various filamentous Conjugatae and Oedogoniaceae, but does not mention any hypertrophy of the host.

Germinating zoospores have been observed in this species in which the germ tube may become directed away from the host and develop a mycelial tube 15–20 μ in length.

RHIZOPHIDIUM MINUTUM

This species was collected on *Spirogyra varians* in a pool beyond Forest Home, N. Y. (about one mile from Ithaca), April 23, 1895. It frequently accompanies Lagenidium, but also occurs independently of it. The zoosporangia are sessile, very small, obpyriform or flask-shaped. Thus the species belongs to the *longata* section of the genus. The zoosporangia are 5–6 μ in diameter. At the base are a few slender rhizoidal threads which extend a short distance in the host cell content. The form of the zoosporangium thus presents a broad and prominent apical papilla, the end of which becomes gelatinized at maturity of the zoospores, forming a circular exit pore. The zoospores are few in number, two to four in the cases observed. They measure 2.5 μ in diameter, and are provided with one cilium and a prominent oil drop.

FIG. 4.—*Rhizophidium minutum* Atk. Elongated zoosporangium, rhizoids, and zoospores.

Rhizophidium minutum, n. sp.—Zoosporangia obpyriform or flask-shaped, broadly papillate, 5–6 μ in diameter, sessile with a few slender rhizoidal filaments

at the base in the host cell. Apex opening by a single pore. Zoospores two to four in a zoosporangium, oval, uniciliate, with a single oil drop, 2.5 μ in diameter.

On *Spirogyra varians* in the vicinity of Ithaca, N. Y.

Zoosporangiis obpyriformibus, 5–6 μ latis, apice una lata papilla, basi filamentis brevibus radiciformibus. Zoosporis ovoidis, 2.5 μ, una guttula hyalina et cilio simplici attenuato praeditis.

Lagenidium rabenhorstii[5]

This species was first found in a species of Spirogyra in a stream of slow-running water in the valley south of the city of Ithaca. It has since been found in various species and appears to be quite common. The fungus attacks the vegetative cells and those just about to conjugate. The zoospore enters the cell wall by a small perforation, the slender entrance tube growing usually nearly to the center of the host cell, where it enlarges to the size of the vegetative thread of the parasite. The general course of the threads is parallel with the axis of the spirogyra cell, though frequently tortuous and curved, with here and there short branches. The threads are usually stout, varying in diameter from 3 to 8 μ. At the ends of the cell of the host they curve around and frequently extend back to the other end, one to three and four threads thus lying in a single cell. The protoplasm possesses numerous highly refringent granules and there are also vacuoles at short distances. In other cases the thread may be strongly curved, or even coiled at various points within the end of the cell.

The chromatophores of the spirogyra are broken down and usually adhere to the threads of the fungus here and there, giving portions of them a green appearance in the early stages, and later a greenish brown as the chlorophyll becomes more and more disorganized. All traces of the chlorophyll at length disappear, and the fungus, lying in a disorganized mass of transparent protoplasm, is then seen distinctly throughout its entire length. In other cases the fungus thread may be permanently soiled by the brownish matter of the disorganized chlorophyll.

The exit tubes are developed from the ends of the threads, or from the ends of the short lateral branches, or from the side of the main

5 Zopf, W., Ueber einen neuen parasitischen Phycomyceten aus der Abtheilung der Oosporeen. Bot. Verein Prov. Brandenburg **20**:77–80. 1878. See also, Zur Kenntniss der Phycomyceten (Zur Morphologie und Biologie der Ancylisteen und Chytridiaceen). Nova Acta Kais. Leop.-Carol. Deutsch. Akad. Naturf. **47**:143–236. *pls. 12–21.* 1884.

thread itself, in the latter cases usually arising from the outer convexity of a curved portion of the thread. The exit tube is about 2 μ in diameter and projects but a little way outside of the wall of the host. The tube develops quite rapidly. In observations on cell cultures where I have been watching for the migration of the protoplasm through the exit tube, forming ones have been observed to develop in two to four hours time. The end of the tube becomes gelatinized and the protoplasm moves in a quite rapid stream through the tube, the small spherical mass of protoplasm at the mouth of the tube growing rapidly in size until all the protoplasm has passed through, which takes only a few seconds. In a few moments rotary motion begins, slow at first. Soon the constriction of the mass occurs in lines over the surface, in such a way as to divide the surface into 2–8 portions according to the size of the mass and the number of the zoospores to be formed. In this species I have not observed any inclosing membrane, nor any evidence that such a membrane exists, though ZOPF (*l. c.*, p. 149, 1884) describes and figures one. The constriction goes on from the surface toward the center, and soon the cilia are developed, their slow waving aiding in the slow but perceptible oscillatory motion of the mass. Plastic movements of the mass and the developing individuals also accompany the other movements. When division has become nearly complete, small portions frequently become constricted from the ends of the ovate to reniform individuals, the constriction becoming deeper and deeper, until in many cases the small portion becomes separate from the larger mass (*fig. 5, C*). These can be seen to be separate from the larger ones by the gliding motion of the zoospores over each other, and over the smaller bodies. The movements become more and more active, occasionally one zoospore pulling strongly in

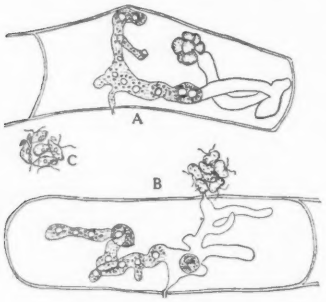

FIG. 5.—*Lagenidium rabenhorstii* Zopf. *A* thallus and prosporangia, which are sections of the thallus; *B* zoospores forming at the apex of the exit tube; *C* zoospores and small particles separated from them.

one direction and separating itself from the group by several millimeters; then it is drawn back again to its fellows as if by a retractile cord; then another will separate in the same manner, only soon to be drawn back again. This pulling away and returning becomes more and more accentuated each time, until finally one of the zoospores makes its permanent escape, leaving the others to struggle still for freedom. One by one, or two or more at a time, they escape in this manner and whirl away. This mode of escape would indicate that there is no inclosing membrane.

Before the escape of the zoospores, in cases where small portions of the zoospores have become separated, they may fuse or conjugate with the large ones again, but whether with the same one from which they were separated or with a different one could not be determined, since the gliding and rotary movement of the individuals and the mass would prevent absolutely the following of the separate ones through their various evolutions. DeBary[6] (p. 37, 1881) first observed a separation of portions of the protoplasm and their fusion again with the parent masses in the formation of the oospores of the Saprolegniaceae, and it has since been observed in the oospores by Hartog[7] (p. 24) and Humphrey[8] (p. 90). It was first observed in the zoospores of the Saprolegniaceae by Rothert[9] (1887, also p. 322, 1890; see also Hartog, *l. c.*). When fusion did not take place before the zoospores made their escape from the group, the smaller portions would not then fuse with the larger ones, even if they came by accident in contact with them, so far as I was able to observe. In other cases the small portions of protoplasm might become partially separated from the larger ones, and the zoospores escape with a minute appendage attached either at one of the extremities or upon

[6] DeBary, A., Untersuchungen über die Peronosporeen und Saprolegnieen und die Grundlagen eines natürlichen Systems der Pilze. Beitr. Morph. u. Phys. der Pilze **4**:1–145. *pls. 1–6.* 1881.

[7] Hartog, M. M., Some problems of reproduction. Quart. Jour. Micr. Sci. **33**:1–79. 1892.

[8] Humphrey, J. E., The Saprolegniaceae of the United States, with notes on other species. Trans. Am. Phil. Soc. **17**:63–148. *pls. 14–20.* 1893.

[9] Rothert, W., Die Entwickelung der Sporangien bei den Saprolognieen. Beitr. Biol. Pfl. **5**:291–349. *pl. 10.* 1890. This paper first appeared in Polish in the Proceedings of the Cracow Academy **17**:———. 1887.

the more convex side, where it gave the zoospore the appearance of being a "hunch back." Such zoospores would perform very curious evolutions in their vain efforts to throw off the offending appendix, but in no case observed did this separation take place after the zoospores had separated from the group at the mouth of the exit tube. The number of zoospores from a single zoosporangium is 2–8, as stated above, or possibly a few more in some cases, the number depending upon the size of the sporangium.

In one case observed, where the zoospores seemed to be rather sluggish in their development and the movements not so active, the form was not so pronouncedly reniform, but more nearly oval, and after separating very little active movement took place, the individuals soon rounding off as they do when ready to germinate. Two such zoospores soon came in contact and immediately fused into a larger one, the fusion taking place in about ten seconds. The further fate of these zoospores was not followed. This fusion may be due to the peculiar conditions of environment, perhaps to the want of fresh water in the limits of the cell culture. From some observations made on the developing zoospores under similar conditions, it appears possible that it may be due to the variations of tension existing between the individuals and the original mass of protoplasm, the tension of the entire mass being directed toward keeping the mass intact and the tension of the individuals tending to separate the masses into smaller individuals. Two cases were observed which had progressed to some extent toward the formation of the zoospores. In one case there were two zoospores forming and in the other case four zoospores were forming from the spherical protoplasmic mass, which had collected at the end of the exit tube after passing from the sporangium. In each case the zoospores were about one-third formed. The preparation had been in the cell culture for two days and the water had been replenished a few times, as it had partially evaporated, by running fresh water under the cover from the edge, the cell cultures being made simply between the cover glass and the glass slip and not in a ring-cell, or VAN TIEGHEM cell. The oxygen thus accessible to the organism was very small, though this did not seem to hinder the development during study, if water were added every few moments, as would be necessary when the preparation was not protected in a

moist chamber. While these two developing groups of zoospores were under observation, the water, which had not been changed for some time, slowly evaporated, so that a portion of it was removed from under the cover. At this time it was noted that the dividing protoplasmic mass, where there were two forming zoospores, was fusing again, and soon the mass was spherical, with no sign of the division which up to this time was quite marked, and showed all the phenomena of movement and formation of cilia which accompany the normal development of the zoospores, except that the movements were not so active. Very soon also the four forming zoospores in the other group were fusing and the movements had likewise ceased. The fusion in this case also continued until there was no trace of the forming zoospores, the mass was again spherical, and movement had ceased.

Thinking this might be due to the want of fresh water, some was quickly run under the cover glass, and the observation was renewed. The smaller protoplasmic mass burst on the absorption of the water, so great was the tension, but the larger one soon began slow rotary movement again and the constrictions appeared a second time, indicating the formation of four zoospores. This time the division proceeded, accompanied by all the phenomena of the formation of the zoospores noted in the normal cases, until the zoospores were complete and whirled away.

This would suggest that there were two opposing tensions in the formation of the zoospores, one individual and under normal conditions the stronger, and the other belonging to the mass and the weaker. When the conditions favorable for the formation of the zoospores ceased, the individual tension lessened to such a degree that it was lower than that of the mass, and the separating smaller portions were drawn together again in the common larger mass. These two opposing tensions might possibly explain the peculiar behavior of the zoospores when they still remain closely associated in the group, now partially separating and again coming close together, continuing this process of coquetting until the individual tension is strong enough to free them, in the case of those where there does not seem to be any inclosing membrane. The tension of the entire mass may possibly be somewhat similar to the force called adelphotaxy

by HARTOG[10] (p. 216) in the case of the escaping zoospores of Achlya and Aphanomyces.

In some of the cell cultures in which numerous zoospores of Lagenidium were developing, there were several sun animalcules (*Actinophrys sol*). Some of these were so full of zoospores which they had caught, that they appeared to be a rounded collection of the zoospores, the convex outer portion of the zoospore only showing, and the entire surface of the Actinophrys appearing like a piece of mosaic made up of these unfortunate creatures. The delicate arms or rays of the Actinophrys radiated for a distance of 20–30 μ, and in a large number of cases which were observed, whenever a zoospore passed within reach of these rays, no matter how swiftly it was moving, it was suddenly paralyzed and halted; then the reniform shape gradually became spherical and it was slowly drawn by "invisible threads" to the body of the Actinophrys and slowly wedged its way between those which had preceded it.

LAGENIDIUM AMERICANUM

Another species of Lagenidium, which seems to differ from any described, was found in various species of Spirogyra (*S. varians*, *S. calospora*, and *S. insignis*) collected in a pool beyond Forest Home, April 11, 1895. It is parasitic in the zygospores and conjugating cells of the spirogyras, but has not been observed to develop in the vegetative cells. The vegetative phase of the fungus consists of strongly curved and coiled tubes, 4–8 μ in diameter, the size varying so that the contour and diameter of the threads is very irregular. It is profusely branched, and has also numerous short branches. It frequently fills the zygospores so completely that the individual course of the thread can be followed for only a short distance. At maturity the tube, as in the other species, is separated into segments of various lengths by the development of cross-walls, forming the zoosporangia. Frequently there is a constriction at the septum. Numerous zoosporangia are developed in a single zygospore. The exit tubes are slender, measuring about 2 μ in diameter, and of variable length according to the direction in which they emerge from the zygospore and the wall

[10] HARTOG, M. M., Recent researches on the Saprolegnieae. Annals of Botany 2:201–216. 1888.

of its parent cell. If the zoosporangium is located at the periphery of the zygospore near the middle, the tube is quite short. In the case of the zoosporangia which are located at the ends of the zygospores, the length of the exit tube is considerably longer, even when it is directed from the start perpendicularly to the wall of the parent spirogyra cell. In many cases, however, the exit tube from the zoosporangia which lie at the ends of the zygospore starts out nearly parallel with axis of the spirogyra cell, or at some angle between this and the perpendicular. In such cases the exit tube may be very long and tortuous, frequently passing into the adjacent spirogyra cell and finally emerging through the wall of the latter.

The protoplasm, with numerous small highly refringent granules and a number of vacuoles, presents practically the same appearance as in the case of the other species. It passes in a rapid stream through the exit tube and collects in an irregularly spherical mass at the extremity, from which in some cases it soon becomes free and floats to a short distance. In ten minutes from the passage slight rotary movements begin, the mass turning a short distance in one direction, and then in a moment in the other direction, the movement becoming faster and repeated at shorter intervals.

In some cases the cilia can be seen as delicate rays, even before the simultaneous constriction of the mass begins and they are slowly lashing. In other cases they are observed very soon after the constriction of the mass begins which outlines the surface of the individual zoospores. No inclosing membrane was observed, and it seems impossible that it should exist if the cilia can extend for such a distance from the mass. As the division proceeds the reniform shape of the individuals becomes more and more pronounced, and the movements become more and more violent. The individuals divide the time in a quivering motion followed by the oscillatory movements and the gliding over one another until they are apparently separate, now one pulling off a short distance, then returning, and so on, until they make their escape one after another or several at a time. After separating from the group, movement in space is rather slow, as if they were uncertain how to use the freedom gained. The individual oscillates and glides around in various curves, then becomes nearly stationary and quivers for a moment; then, one end remaining

stationary, the other executes in succession several jerks or nods as if striking at something; then it enters upon another series of evolutions. After a short time thus engaged, the activity of the zoospore increases, the field covered by its evolutions becomes larger, and it is lost from the field of the microscope. In some cases which were timed the zoospores were separate in 25–30 minutes from the time the

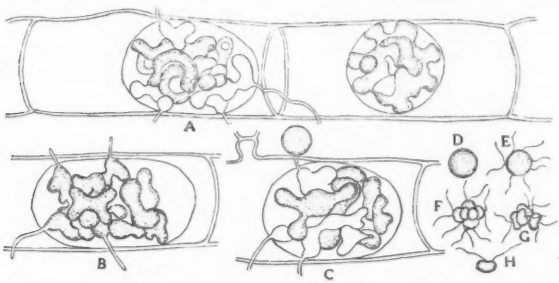

FIG. 6.—*Lagenidium americanum* Atk. in zygospores of Spirogyra. *A*, *B* some empty and some mature prosporangia; *C* from one prosporangium the protoplasm has just issued from the exit tube; *D* plasma sphere or zoosporangium proper free from the exit tube and floating in water; *E* formation of cilia where a zoosporangium is swimming around as a multiciliate spore; *F*, *G* different stages in formation and separation of zoospores, the groups swimming around like a pandorina colony; *H* mature and separate zoospore.

protoplasm passed from the exit tube. In those cases where the plasma vesicle at the end of the tube becomes free and floats away, the cilia develop on its surface, and it at first presents the appearance of a multiciliate spore. Later the divisions occur in such a way as to leave two cilia with each forming zoospore, and the colony then resembles a pandorina colony.

Lagenidium americanum, n. sp.—Plant body in the form of irregular tubes, much branched, curved, and of unequal diameter, confined within the host zygote, 3–8 μ in diameter. Exit tubes slender, short, 2 μ in diameter, varying in length but extending a short distance outside of the gametangium wall, of equal diameter from the point where they pierce the wall of the zygote to the outer free end, thus differing from the exit tubes of *L. entophytum* (Pringsh.) Zopf. Exit tubes arising from the end of hyphae or branches, or from the convex side of curved or enlarged portions. Zoosporangia of varying size, irregular and often branched, formed from segments of the thallus. Plasma sphere at the end of the exit tube often becoming free and floating away (the cilia then forming on the outside of the

sphere), and finally dividing into the individual zoospores, the whole resembling a pandorina colony, but at last the zoospores separating. Zoospores reniform, laterally biciliate, 4–6×5–7 μ.

In zygospores of *Spirogyra varians*, *S. insignis*, and *S. calospora*, Ithaca, N. Y.

Mycelio inequali, curvato, ramulo irregulare instructo, 3–8 μ lato. Zoosporangiis irregularibus, saepe ramulosis. Ostiolo tubiformi tenuissimi aequali, 2 μ lato. Prosporangiis ramulosis sectionibus mycelii formatis. Zoosporangiis vesiculosis, saepe deciduis, ciliis ornatis, ut coenobio Pandorinae natantibus, postremo divisis, zoospores formantibus. Zoosporis fabaeformibus, laterale biciliatis, 4–6×5–7 μ. Hab. in zygosporis Spirogyrae, Ithaca, N. Y.

PHLYCTOCHYTRIUM PLANICORNE

This genus, *Phlyctochytrium* Schroeter,[11] differs from Rhizophidium in the presence of a swelling on the penetration tube, just inside the host cell wall, from which the delicate nutritive rhizoids grow. *Phlyctochytrium planicorne* has been found quite frequently in company with *Lagenidium americanum*, in the cells of *Spirogyra varians*, from the pool beyond Forest Home (near Ithaca). The zoosporangium is a little broader than long, measuring 6×8 μ, and is broadest in the middle. At the apex it is provided with four plain dentate processes around the exit pore. These dentate processes are characteristic of one section of the genus (*Dentigera* ROSEN,[12] SCHROETER, *op. c.*, p. 79). The endophytic bladder-like base of the plant is separated from the epiphytic zoosporangium by a constricted portion at the point where the penetration tube passes through the cell wall of the host. This base is about 3 μ in diameter, and from it radiate several slender branching threadlike rhizoids. No zoospores have as yet been observed. While it very frequent'y accompanies Lagenidium, it is by no means confined to the cells affected by the fungus, being found on other cells also.

FIG. 7.—*Phlyctochytrium planicorne* Atk.

Phlyctochytrium planicorne, n. sp.—Zoosporangium broadly elliptical, 6×8 μ, armed at the apex with four plain teeth. Endophytic vesicle globose, 3 μ in diameter, with several radiating branched slender rhizoids.

On cells of *Spirogyra varians*, often accompanying *Lagenidium americanum*, near Ithaca, N. Y.

[11] SCHROETER, J., in ENGLER & PRANTL, Pflanzenfam. 1[1]:78. 1892.

[12] ROSEN, F., Ein Beitrag zur Kenntniss der Chytridiaceen. Beitr. Biol. Pflanzen 4:253–266. *pls. 13, 14.* 1887.

Zoosporangio ellipsoideo, 6×8 μ, apice 4 cornuis ornato. Cellula inferiori 3 μ lata. Hab. in *Spirogyra varianti*.

PHLYCTOCHYTRIUM EQUALE

This species inhabits the cells of Spirogyra, having been found in the cells of *S. insignis*, collected in the pool beyond Forest Home, April 17, 1895. The epiphytic sporangium and the subsporangial endophytic base are about equal in size, being spherical in form and about 6 μ in diameter. The mouth of the sporangium appears to have two small teeth, which may be the walls of a short canal. From the base of the subsporangial part of the plant are several branching rhizoid-like threads. Zoospores have not been observed.

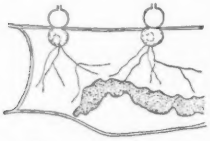

FIG. 8. — *Phlyctochytrium equale* Atk.

Phlyctochytrium equale, n. sp.—Zoosporangium globose, sessile, about 6 μ in diameter. Subsporangial base equal in size, with several long branched rhizoid filaments from its base.

Zoosporangio globoso, sessili, 6 μ lato, basi subsporangiali aequali, rhizoideis filamentibus ramulosis praedito. Hab. in *Spirogyra insigne*.

A few other species have been observed but not studied further than for their identification. They are as follows:

Lagenidium enecans Zopf (p. 154, 1884), in *Stauroneis phoenicentron* and *Cymbella lanceolatum*, in swamps near Freeville, N. Y., May 5, 1895.

Entophlyctis bulligera (Zopf) Fischer (*Rhizidium bulligerum* Zopf, p. 195, 1884), in *Spirogyra insignis* in pool at Forest Home, N. Y., April 17, 1895.

Rhizophidium ampullaceum (A. Br.) Schroeter, on sterile threads of Oedogonium, Freeville, N. Y., May 4, 1895.

Ectrogella bacillariacearum Zopf (p. 175, 1884), in diatoms, south end of Cayuga Lake, Ithaca, N. Y.

CORNELL UNIVERSITY

MITOSIS IN SYNCHYTRIUM

WITH SOME OBSERVATIONS ON THE INDIVIDUALITY OF THE CHROMOSOMES[1]

ROBERT F. GRIGGS

(WITH PLATES XVI–XVIII)

The nuclei of *Synchytrium decipiens*, a leaf parasite of the hog peanut, were shown in previous papers to be derived very largely by amitosis. Several sorts of amitosis were observed, two of which, nuclear gemmation and heteroschizis, differ considerably from the ordinary process of amitosis. The nuclei derived by these direct divisions were shown to be persistent, and some of them were observed to divide by mitosis. This with other facts led me to the conclusion "that the nuclei derived by these processes of amitosis are normal, and that they with their descendants become the functional nuclei of later generations, capable of perpetuating the species" (**5**, p. 134). The purpose of the present paper is to describe the mitoses which follow, to correlate them with the amitoses, and to discuss the theoretical bearing of the facts thus presented.

I would here repeat my acknowledgments to my friend, Professor F. L. STEVENS, of the North Carolina College of Agriculture and Mechanic Arts, who furnished the material used in the investigation and gave the suggestions that originally aroused my interest in the problem. The sections were cut 2–10 μ thick and stained with Haidenhain's iron alum hematoxylin and with anilin safranin and gentian violet.

As in coenocytes generally, all the nuclei in a cyst pass into mitosis simultaneously and divide with equal rapidity, so that all of them are very nearly in the same stage throughout. This fact may be utilized for overcoming one of the most serious obstacles encountered in investigating the cytology of this plant—the difficulty of estimating the relative ages of the different structures in the absence of any

[1] Contribution from the Botanical Laboratory of the Ohio State University, No. XLIX.

indications external to the nuclei themselves—for those cysts which are of critical age usually contain short series of closely connected stages which frequently enable one to assure himself concerning difficult points, such as the formation of the spindle or the origin of the asters. Nuclei of widely different phase in the same cyst are very rare: *fig. 5* is a case where the small nuclei in the cyst were still in the vegetative condition while the large nuclei had passed into spirem; *fig. 35* shows a case where the small nuclei had reached late anaphase while the large nuclei were still in metaphase; *fig. 36* of the previous paper (**5**) is a case where the reverse was true, the small nuclei being in metaphase while the large ones were in anaphase. It would be a matter of great interest to ascertain the mechanism by which the nuclei are kept in phase. While not throwing much light on the nature of the stimulus, the behavior of newly segmented cysts is interesting in this connection. In such a cyst, long before the walls of the zoosporangia appear, while the segments are still separated from each other only by an exceedingly delicate plasmatic membrane, each nucleus has become entirely independent of the others; and one finds vegetative nuclei, metaphases, and telophases in adjacent segments, showing how slight a separation suffices to establish the complete physiological autonomy of the individual nucleus (*fig. 33*).

All of the mitoses of Synchytrium are of the same type. It is true that none of the later nuclei undergo such a shrinkage in volume with its associated peculiarities as occurs preparatory to the primary mitosis (STEVENS **18**), but these phenomena are probably due simply to the enormous size of this overgrown nucleus. It may be recalled that similar peculiarities are usually observed in very large nuclei wherever they are found. CHAMBERLAIN (**1**), for example, was not able to interpret the structures he found in the enormous egg nucleus of Dioon. But aside from these peculiarities of the primary nucleus, the only differences that could be detected were in the size of the spindles, those in the early stages being fairly large, while those in the last mitoses of the sporangium are exceedingly minute (*figs. 32–34*).

In the resting nuclei of Synchytrium the chromatin is all concentrated in a single globular karyosome (*figs. 1, 31*). Except in those cases where vacuoles have appeared on the removal of the chromatin preparatory to mitosis or nuclear gemmation (*fig. 36*), the karyosome

is an entirely irresolvable, deeply colored body, whether stained with hematoxylin or safranin. Besides the karyosome there may be a few deeply staining granules on the nuclear membrane, but there is nothing corresponding to the chromatin reticulum characteristic of the vegetative nuclei of many cells. The only nuclei in which the chromatin approached the condition of a reticulum were located in the degenerating cysts occasionally found.

The spirem

The early prophases of mitosis consist in the formation of the spirem from this compact karyosome. This is brought about in the most direct manner possible. The karyosome first separates into a number of irregular chromatin masses (*figs. 2, 30*); next delicate linin bands appear connecting these granules with the nuclear membrane (*fig. 3*); and along these bands the chromatin granules are distributed over the nuclear cavity, forming the expanded spirem (*fig. 4* from the same cyst as *fig. 2*).

Only a portion of the spirems of Synchytrium, however, are destined to pass into mitosis. A large proportion of them undergo nuclear gemmation or some other form of amitosis. In view of theoretical considerations which will be discussed later, it is of the utmost importance to determine whether the spirems of amitosis are of the same nature as those of mitosis, or whether they are different in kind and present only accidentally an optical similarity. In most cases it is easy to distinguish the two sorts by the cysts in which they occur. In those cysts which are in the prophases of mitosis, the nuclei are in general very nearly the same size and evenly spaced off from each other, while in the amitotic cysts there are small nuclei in groups or clusters as well as the large spirems. Many such spirems have a strikingly different aspect from the mitotic spirems. They are coarser (*fig. 37*), the chromatin granules are very much larger, and the linin strands are short and heavy; whereas the mitotic spirems are characterized by linin threads and chromatin granules of various sizes lying side by side. In the amitotic spirems each of the chromatin granules is evidently simply the karyosome of a small nucleus, for the formation of which the spirem is a preparation. It would thus appear that the two classes of spirems are entirely different from each other, but

unfortunately for this view a very large proportion of the amitotic spirems are intermediate, or so closely resemble the mitotic spirems that they can be distinguished from them only by the character of the cyst in which they occur (*fig. 41, a, b, c*). It seems impossible therefore to determine certainly whether these two classes are distinct or whether they are of the same nature.

The origin of the spindle

In those cells where the spindle is intranuclear, there seem to be two types of spindle formation. In the first type, which occurs in the ascomycetes (e. g., Phyllactinia, HARPER **8**) and brown algae (e. g., Fucus, YAMANOUCHI **21**), the centrosomes are permanent organs of the cell and form the spindle by moving around the nucleus so as to include the chromosomes between them, organizing the spindle by the union of those astral rays which penetrate the nucleus in a manner very similar to that prevalent among animals. This process is very conspicuous and has been observed and figured by numerous investigators. In the other type, which occurs in the oomycetes (e. g., Saprolegnia, DAVIS **3**; and Albugo, STEVENS **16**), centrosomes are either small or absent in the early stages; to this type the spindles of Synchytrium belong. Here the determination of the origin of the spindle is very difficult. This probably accounts for the fact that very few of those who have figured such mitoses have given a series of figures of the prophases complete enough to throw much light on the formation of the spindle.

When first seen, the spindle of Synchytrium, which is thrown directly across the cavity, is not distinguishable by its staining reaction or otherwise from a strand of the spirem (*figs. 6–8*). It is therefore difficult to be certain just when the spindle appears or what it comes from, but it gives every indication of being differentiated from a spirem strand. Very soon, however, it becomes sharply pointed and quite different from the spirem, which now begins to be drawn in around its equator (*figs. 9, 10*). Contraction continues and more definite connections between the chromatin and the spindle fibers are established (*figs. 11, 12*). In this condition the spindle often appears bipolar (*fig. 9*), which emphasizes the similarity of the linin strands and the spindle fibers. By further contraction this much shortened

spirem is converted into the four chromosomes of metaphase (*figs. 14, 15*). Very frequently, however, the contraction is so pronounced that the individual chromosomes cannot be made out in the chromatic mass at the equator (*fig. 13*).

Some spindles show nucleoli lying in the nuclear cavity beside the spindle; in others no nucleolus is present. No difference was observed between the spindles with nucleoli and those without; but it was observed that in any given cyst all of the spindles were alike in this respect—either all had nucleoli or all lacked them. In rare cases more than one of these bodies were present. The nucleoli remain beside the spindle till after the daughter nuclei have separated in telophase (*figs. 22, 29, 33*), when they disappear.

As soon as the spindle is formed, the nuclear membrane with the small chromatic granules which are imbedded in it begins to disappear (*figs. 10–15*), soon leaving the spindle free in the cytoplasm. As is usual with the intranuclear spindles of fungi, the metaphase in which the chromatin is all concentrated at the equator, if we may judge from the frequency with which it is observed, is of long duration. This is in contrast with the mitoses of the higher plants, where good metaphases, far from being the commonest, are observed less frequently than other stages.

Anaphase and telophase

The spherical chromosomes are pulled away from each other in the usual manner by the fibers of the spindle, which are exceedingly heavy (*figs. 15, 17*). In early anaphase, figures stained with anilin safranin and gentian violet show the chromosomes red and the spindle violet but in hematoxylin preparations the chromosomes are difficult to differentiate from the spindle fibers. This difficulty increases in late anaphase until it becomes impossible to distinguish chromatic from achromatic structures, even when stained with the safranin-violet combination. When the chromosomes pull apart, they remain connected by heavy fibers similar to those by which they were pulled away from each other (*fig. 18*), which persist long after the chromosomes become lost in the condensed mass at the poles (*fig. 19*). These deeply staining fibers are very conspicuous and give a characteristic appearance to side views of anaphases, which somewhat resem-

bles the daughter stars formed in mitoses where the chromosomes themselves are elongated. These fibers may be used to determine the chromosome number, since each one of them is connected with a chromosome. This can be done more easily in this stage than in metaphase, because the fibers are larger than the chromosomes and because they spread apart as they separate, sometimes giving views of all four at once, while in any other stage the full number can be seen only from polar view (*fig. 16*).

These radiating bands of fibers are now drawn in, leaving the two poles connected only by a central band (*figs. 20, 22*), which becomes considerably attenuated as the two chromatic masses move farther and farther apart. The daughter nuclei become separated by distances considerably greater than the original length of the spindle. In moving apart one or both of them frequently swerves from the axis of the spindle, so that the connecting fibers meet at a considerable angle (*fig. 33*). As was indicated in the paper on the reconstruction of the nucleus (GRIGGS 4), this habit makes it almost impossible to associate the daughter nuclei in pairs after the wisps of spindle which point to the original position of the equator have disappeared, especially since the wide separation greatly lessens the chances of securing both in the same section. For this reason all figures of stages after this period are drawn from only one of the daughter nuclei. After the spindles have broken in two, the telophases are very inconspicuous objects; though they stain deeply, the chromatin is concentrated into so narrow a space that they are practically invisible under any but the highest power (*figs. 23, 35b*).

The aster

As previously reported by STEVENS, KUSANO, and myself, there are no granules, radiations, condensations, or any other indications of the presence of centrosomes at the poles of the spindle during prophase or metaphase. But about the time the two halves of the spindle separate, radiations begin to emanate from the chromatic masses. At first so delicate as to be on the very limit of visibility, the aster rapidly increases in prominence until it becomes exceedingly conspicuous, clearly visible under a magnification of forty or fifty diameters. At first the radiations may appear to emanate from the

center of the chromatic mass (*figs. 24, 25*), but very soon it is evident that the focus is beyond the condensed chromosomes (*fig. 24*). As the rays increase in strength the focus is shifted, until there is a considerable interval between the center and the chromatin (*fig. 27*). In this stage, since the chromatin is condensed to its minimum volume, the aster is so very much more prominent than the chromatin that the latter is likely to be overlooked altogether, making it appear that the cyst has no nuclei, but only asters! The chromatic mass soon enlarges, however, and rounds off into the spherical karyosome of the resting nucleus (*figs. 27, 28*). Up to this stage the chromatin lies suspended in the cytoreticulum, without any apparent relation to it. The appearance of the karyosome, however, marks the resumption of definite relations of nucleus and cytoplasm in the formation of a vacuole around the chromatin (*fig. 28*). This vacuole is at first bounded only by the meshes of the cytoplasm, but soon the rays of the aster bend around it and form the heavy nuclear membrane which incloses it (*figs. 29, 30*), as previously described by KUSANO (**10**) and myself (**4**). When the nuclear membrane is complete, the aster gradually disappears; the rays first become much more numerous and finer; the center gradually becomes diffuse and stains less deeply (*fig. 4*); and finally the aster is transformed into a condensation of cytoplasm as previously described (*figs. 31, 20, 3*).

During the disappearance of the aster, however, the nucleus usually enters into the prophases of the next division, so that only seldom is a cyst found where the nuclei are still in their vegetative condition when the asters are in their last stages. On the other hand, it is not at all unusual to find that the new spindle has already formed, before the aster has disappeared, and in rare cases the anaphase of the succeeding division (*fig. 20*) may be reached before the old aster is completely gone. The disappearance of the aster probably occupies a period of somewhat definite length, while the rapidity with which the mitoses succeed each other varies greatly in different cysts. It was this lack of correspondence between the cycles of astral and nuclear metamorphoses that made it necessary in the former paper on this subject to state some conclusions provisionally that may now be positively established.

From their function of forming the nuclear membrane, the asters

of Synchytrium have been named *karyodermatoplasts* by KUSANO (13). Although somewhat long, this term may be useful in alluding to the *function* of the asters, but there seem to be certain objections to its use as the name of a structure. First, the structure so designated is so variable that it is difficult to define it. Sometimes the aster is single, sometimes double or triple (*fig. 2*); sometimes it has one clearly defined granule at the focus of the rays; more often there are several such granules more or less eccentrically placed; or there may be none at all. Sometimes there is an elongated band which bears radiations all along its length, like the blepharoplast of a cycad. It is evident that the term karyodermatoplast can be defined only by its function, while such a term should rest on a morphological basis. Second, though the aster, so far as observation has yet indicated, serves only to reconstruct the nuclear membrane, that function alone does not seem to the writer adequate to account for its enormous development in Synchytrium. This is more apparent when one recalls the fact that in heteroschizis and nuclear gemmation the membranes of the daughter nuclei are formed without the intervention of any such structure. I have preferred, therefore, in the present discussion to employ the descriptive term aster for the structure in question, without committing myself to its significance. The question of the radiate structures in Synchytrium and their homology is one of very great interest and deserves consideration in a separate paper.

The chromosome number

Inasmuch as most of the nuclei or their ancestors have been derived by amitosis, as has been previously shown, the determination of the number of chromosomes in Synchytrium becomes a matter of much more importance than in organisms where the orderly sequence of mitosis has not been interrupted. On this account especial care has been taken to insure accuracy of observation and interpretation. Upward of 500 slides have been used in the study. Altogether several hundred mitotic cysts with many thousands of individual spindles have been observed. Of these only the most favorable were used for basing the conclusions. The location of the cysts containing these most favorable spindles was recorded by vernier readings of the mechanical stage and filed in a card catalogue, so that they could be

reexamined in rapid succession as frequently as desired. The favorable cysts so listed number about fifty; most of them contain several hundred spindles.

All of the results obtained were not exactly concordant, but I shall give the observations on which the conclusions were based that the reader may be enabled to judge of their soundness for himself. In all but two of these favorable cysts there were constantly four chromosomes. In these two, however, the number of chromatin bodies was certainly more than four (*fig. 42*). But while the chromosomes are all of the same size, some of these bodies were smaller than chromosomes and had the appearance of masses of chromatin from the late spirem which had not yet fused together into the compact chromosomes of metaphase. Inasmuch as the spindles were undoubtedly newly formed, this is probably the correct interpretation, especially as there seemed to be in some cases faint wisps of spirem remaining about the equator of the spindle. But whether these supernumerary chromatin bodies are to be explained on some such basis or whether they are actual irregularities in the number of chromosomes, I am of the opinion that they do not seriously weaken the conclusion that the chromosome number is four. Although it is unusual for a writer to state difficulties of this sort as frankly as has WILSON (**20**) in his study of Metapodius, seeming discrepancies in the number arising from one cause or another are, I believe, frequently met with in efforts to count chromosomes.

In all of the other cysts studied there was no deviation from the constant number four. On all of the spindles the chromosomes were placed at angles of about 90°, so that two, three, or four of them were visible, according to the angle of observation (*figs. 14–19*). In the very much larger number of cysts whose spindles were not favorable enough to permit exact counting, there were no indications of a different number. It should be noted also that four is an exceedingly easy number to count, for one can *see* four without counting them one by one as is necessary for a larger number. In thus maintaining a constant number of chromosomes the writer is supported by the only other published accounts of the chromosomes of Synchytrium. STEVENS in his paper on the primary mitosis (**18**) states (p. 413) that "they are probably four in number, although we do not assert

this with certainty," and in his second paper he shows spindles with the same number of chromosomes. KUSANO (**10**) reports definitely five chromosomes in *S. puerariae*.

The probability that the results thus obtained are accurate is very greatly increased by the fact that in Synchytrium there is no differentiation into soma and germ plasm. Every nucleus becomes either directly or through its descendants the nucleus of a spore, which, if successful in entering its host, becomes the large nucleus of a primary cyst. If a variation of the chromosome number occurred at any point, it would, on the individuality hypothesis, be perpetuated indefinitely, affecting all the nuclei of later generations; whereas in the higher plants and animals a variation in the somatic nuclei would be lost with the death of the individual organism in which it occurred. It is clear, therefore, that if a variation in the number of chromosomes should occur only once in the repeated direct divisions through which the nuclei of the spores have been derived, it would affect all the nuclei of the next generation. Thus, though the nuclei of the later stages of the parasite are so small that an irregularity in the chromosome number might not be detected, yet in the large nuclei of the succeeding generation it could not escape observation. Amitosis plays so important a part in the formation of the nuclei that if chromosome variations occurred in only one per cent. of the direct divisions, the nuclei of the whole parasite would in the course of a few generations become so irregular that it would be impossible to recognize the original number of chromosomes when it did occur. It may also be remarked that an irregular reduction in the number of chromosomes, such as might be expected in the amitoses of a non-sexual organism like Synchytrium, could lead, when repeated in indefinite series, to only one result—all the nuclei would finally have only one chromosome.

On the individuality of the chromosomes

Modern cytology may be almost said to be built around the theory of the individuality of the chromosomes, and certainly no other hypothesis has borne so much fruit in valuable results as this. Without it the complicated process of mitosis would seem to lack significance, and the doubling and reducing of the chromosomes in

fertilization and reduction would be well-nigh meaningless. For its support it has had, besides the constancy of the chromosomes in the species, various observations on nuclei which were known to contain either more or less than the normal number of chromosomes.

The great difficulty in the way of the theory has always been that the chromosomes apparently lose their individual existence during the vegetative phases of the nucleus. In many cases where the nuclei are rapidly dividing the chromosomes do not, however, entirely lose their individuality between the successive divisions, but divide and reproduce themselves directly from the compact condition of mitosis. These cases have been the evidence by which the vegetative period of the nucleus was bridged over by the individuality hypothesis, for there is an unbroken series of intergradations from this condition to that in which the chromosomes have completely lost their morphological identity, and one would not suppose that nuclei in one condition differed fundamentally from those in the other. Accordingly, basing their statements on observations of nuclei in which the individual chromosomes could be traced, with more or less certainty, from mitosis to mitosis, several writers have asserted confidently that the individuality of the chromosomes is maintained through all conditions of the chromatin, whether visible or not.

But the transformations of a set of chromosomes which, after they are once formed, persist and divide are not necessarily equivalent to the formation of a new set from a diffuse reticulum. If the chromatin reticulum gave rise only to the chromosomes, the analogy might be closer, but when, as frequently happens, large amounts of chromatin are cast out into the cytoplasm as nucleoli, microsomes, or in mass, it is evident that the succeeding differentiation of the chromosomes involves factors different from those entering into the mere transformation of a chromosome which persists *intact* through whatever metamorphoses it may pass. This may be illustrated perhaps by comparing the chromosomes to metal rods which remain distinct from each other when cold, but as the temperature approaches the melting point become soft, lose their shape, and partially fuse together, though each retains its distinctness and can be reclaimed unchanged on cooling. But after they have once melted together, all this is changed, and they can be regained from the homogeneous flux only by

making them entirely over again. It seems quite possible that in the diffuse vegetative reticulum the chromosomes are as completely fused as the molten metal, and until we know more than we do now concerning the nature of the reticulum, this alternative possibility should be kept before us. The analogy of the molten metal is more suggestive in cases like Synchytrium, where the chromatin is partially or completely concentrated in a karyosome from which part or all of the spirem is directly derived. Although cytologists have as a rule paid but little attention to their behavior in mitosis (cf. WAGER 19), such structures are common occurrences in many plants and animals.

If we may infer safely, as I believe we may, that the chromosomes of *Synchytrium decipiens* number constantly four, it becomes a matter of primary interest to determine how that constant number is maintained in all of the amitoses through which the nuclei pass. It has long been assumed that the principal if not indeed the sole function of the complicated process of mitosis was to insure exactly equal division of the chromatin between the daughter nuclei. In view of the fact, however, that the majority of the nuclei of Synchytrium are derived by some form of amitosis either directly or through their ancestors, and yet maintain the number of their chromosomes constant, it is evident that mitosis is not necessary to maintain this number.

The possibilities of an exact mechanical division of the chromatin are somewhat different in the different varieties of amitosis. In heteroschizis there occurs a metamorphosis of the nucleus suggestive of that of mitosis. In the loss of the nuclear membrane and the apparent cessation of interaction between the nucleus and the cytoplasm there may be a pause during which the chromatin is divided granule by granule in such a way that the karyosomes of the daughter nuclei are furnished with exactly equal chromatin content, just as is visibly accomplished by the fission of the chromatin granules or the chromosomes in mitosis.

Likewise it is not inconceivable that the karyosome may be divided in a similar manner when it is broken up preparatory to nuclear gemmation, though in this variety of amitosis there is no loss of the nuclear membrane or other indication of a pause in metabolism.

An exact equational division of the chromatin under such circum-

stances would be something of a novelty, but it would not necessarily involve functions of the chromatin differing fundamentally from some with which we are more familiar. Whenever the chromatin is withdrawn granule by granule from a karyosome (nucleolus) preparatory to mitosis, we must suppose, on the individuality hypothesis, that the granules come out of the nucleus as they went in, properly sorted, so that each granule is taken into that chromosome from which it came. On the assumption of an equational division in heteroschizis and nuclear gemmation, we should have to assume, in addition to this well-known property of the chromatin granules to sort themselves out from the apparently homogeneous mass of chromatin of the karyosome, only the further property of reproducing themselves while yet within the karyosome as they ordinarily do in the spirem. This is perhaps not too much to ascribe to the chromatin, but even such an addition to our theory would add a very large field for speculation where the conclusions, with our present methods, could never be checked by observation.

But when nuclear gemmation takes place in spirem two alternative possibilities are presented, the choice between which depends on the nature of the spirem involved. If the spirem is an entirely different structure from the mitotic spirem, it would be rational to suppose that each small karyosome, i. e., each granule of the spirem in nuclei such as *fig. 37*, contains four chromosomes derived by an equational division of the mother karyosome as in the previous cases. But if the amitotic spirem is like the mitotic, the situation is entirely different, for we know from its later history the composition of the mitotic spirem. It contains altogether only four chromosomes, which are arranged serially, and any small part will contain chromatin from only *one* chromosome, if a small part of such a spirem is extruded and forms an independent nucleus. Therefore, it is evident that its chromatin is derived not from all four but from only one chromosome. It is then a matter of great importance to determine the constitution of the spirem, but unfortunately, as stated above, the evidence on this point is somewhat ambiguous, and a definite decision in favor of either alternative seems impossible.

Certain features of nuclei dividing by constriction, however, seem to throw some light on this matter. When amitosis by constriction

occurs, the nucleus develops lobes and divides up into a number of daughter nuclei varying from two to a dozen or even more (*fig. 41, a, c*). One cannot tell by observation whether a nucleus is about to divide into two or many daughters, for there is no apparent segregation of chromatin into parts corresponding in number with the number of small nuclei to be formed. Nor are the small nuclei necessarily equal in size or chromatin content. Sometimes the variations are very great; thus *fig. 36* shows a cyst in which the primary nucleus has divided, leaving the nucleolus undivided in one of the daughters, while the other and smaller daughter nucleus has constricted or budded again to form a relatively minute nucleus at one side. It is difficult to believe that these three nuclei had their origin in equational divisions of the chromatin previous to the actual constriction of the mother nuclei. All appearances go to indicate rather that the factors controlling the division of these nuclei are entirely disconnected from the behavior of the chromatin, and favor CHILD'S hypothesis (**2**) that amitosis is merely a physical process. But whether this be the case or not, nuclei so formed are normal and sometimes pass into mitosis. When this happens they show the four chromosomes characteristic of the species (*fig. 35, a*). More commonly, however, amitosis by constriction is but an incident in the division of the chromatin, for the constricted nuclei are soon completely converted into groups of small nuclei by gemmation (see **6,** *fig. 3*). Since the constitution of these nuclei is of great importance in this connection, I have introduced here a series of three drawings of nuclei from the same cyst. *Fig. 38* shows a case of amitosis of the type most commonly observed, though it is not common in Synchytrium; in *fig. 39* the two nuclei are completely separated but still touch each other; in *fig. 40* one of the granules similar to those on the periphery of the other nuclei has formed a small nucleus by gemmation. The individuality hypothesis leads us to rather startling conclusions regarding the constitution of these nuclei. Each of the large nuclei must have four chromosomes. And since each of the granules on the periphery has the power of organizing a new small nucleus with four chromosomes, it also, if the chromosomes have a material continuity from generation to generation, must contain four chromosomes. In other words, the large nuclei must at one and the same time have four and $n \times 4$ chromo-

somes. In these nuclei the small karyosomes number 4, 5, 6, 7. The respective nuclei must therefore have 16, 20, 24, 28 chromosomes, and still have no more than four!

There seems to be no option but to conclude that in Synchytrium there is no morphological continuity of the individual chromosomes, nor is there any definite set of chromatin granules of whatever size which are passed on intact from nucleus to nucleus. The number of chromosomes seems to be a physiological rather than a morphological constant. Not the chromosome but the nucleus itself seems to be the morphological unit of the lowest order. Apparently any mass of chromatin that is capable of organizing a nucleus at all, i. e., any particle of nuclear matter that is able to continue life and reproduce itself, whether it originally contained portions of all of the chromosomes or of only one of them, will preserve the characteristic number of chromosomes along with the other hereditary characters of the species.

Although such a hypothesis seems to be required to explain the phenomena of nuclear division in Synchytrium, it would not be wise to attempt to reach any decision as to its general applicability at this time. In proposing it to account for these features of Synchytrium, the writer is not at all oblivious of the enormous amount of evidence which has been heaped up in recent years in support of the individuality of the chromosomes. The occurrence of protochromosomes in the nuclei of many cells (OVERTON **14**); the persistence of supernumerary chromosomes in cases of polyspermy, etc.; and the remarkable size and shape differences among the individual chromosomes such as have been discovered in animals, notably by MCCLUNG and MONTGOMERY, and recently in plants by SCHAFFNER (**15**) and Miss HYDE (**9**), along with many other observations of a similar nature, are facts which give very great weight to the hypothesis of the individuality of the chromosomes. Nevertheless, it must be remembered that almost all of the evidence in favor of the individuality hypothesis is of suggestive rather than demonstrative value. This was clearly recognized by all observers until about five years ago, but since that time the amount of such evidence has increased to such an extent that cytologists have been much less cautious than before in using the hypothesis. The renaissance of MENDEL'S law so favored the general

acceptance of the chromosome hypothesis by making it highly desirable on theoretical grounds to find the material bearers of hereditary units which the law seemed to demand, that practically all opposition to it was swept away, and it has come to be the groundwork on which present-day cytology has been constructed. If then the basis on which the theory rests is in any degree unproven, it is well for cytologists to keep that fact clearly before them, that they may be guarded against making any false steps in blind adherence to an insecure hypothesis or of failing to notice any evidence which might throw doubt on the validity of their theory.

The writer for his own part, however, has no intention of discarding the whole hypothesis of chromosome individuality on the basis of these observations on Synchytrium, or on the similar results obtained by CHILD (2) from studies of representatives of nearly all the great animal phyla. The conflict of evidence for and against the hypothesis seems clearly to require us to keep the case open for the present. It is as tending to promote such a judicial attitude of suspended judgment among cytologists rather than as overthrowing the whole theory that the writer would have his results received.

Summary

The mitoses are all of the same type and occur simultaneously throughout the cyst.

The spirems of amitosis are frequently indistinguishable from those of mitosis.

The spindle is of the oomycete type, without centrosomes.

The asters first appear as emanations from the condensed chromatic mass of telophase, but quickly separate from the chromatin and their rays form the nuclear membrane.

The number of chromosomes is constantly four.

In some sorts of amitosis an equational division of the chromatin previous to the division of the nucleus is possible, though there is no evidence of such a process.

In other varieties of amitosis an equational division of the chromatin does not seem to be possible, but rather direct division gives some indication of being merely a physical process as suggested by CHILD.

Nevertheless, nuclei known to be derived by amitosis show four chromosomes.

It is therefore concluded that in Synchytrium there is no morphological or material continuity of the chromosomes from generation to generation of nuclei; but that the chromosome number is a physiological constant, like the other hereditary characters of the species.

COLUMBUS, OHIO

Addendum

After the foregoing paper had been completed and submitted for publication, KUSANO's "Contribution to the cytology of Synchytrium and its hosts" (Bull. Col. Agr. Imp. Univ. Toyko **8**:80–147. *pls. 8–11*. 1909) reached me. KUSANO's paper, for the most part, is based on observations of *S. puerariae*, which seems to be remarkably similar to *S. decipiens*, which he also used for comparison. He gives much space to the metamorphosis of the nucleolus (karyosome), which is unusually favorable for study in Synchytrium, showing that all the elements derived from the mother nucleus are concentrated in the karyosome of the daughter nucleus from which they are later withdrawn. He figures the prophases and metaphases of the primary mitosis, confirming for the most part STEVENS' observations, but like him failing to find the anaphases and telophases, which for some reason seem to be very difficult to observe. He then devotes considerable space to the secondary mitoses, obtaining results similar to those of the present paper; but his account of the prophases differs considerably from that of the present writer. His series of figures, however, is not complete at this stage, and he admits (p. 102) that he had not been able to follow the formation of either the chromosomes or the spindle. Again, in the telophase there is a wide gap between his *figs. 55* and *56*, during which the aster is developed, a process about which he was left to conjecture (p. 127), incorrectly supposing that it originates from the cytoplasm. In addition to the method of segmentation by cleavage furrows described by HARPER, he reports a second method in which the sporangium walls are precipitated by the cytoplasm as in the endosperm of the higher plants. This form of segmentation occurs also in *S. decipiens* and, according to my observation, is more common than that described by HARPER.

KUSANO saw and figured the clusters of small nuclei due to various sorts of amitosis as described in my former paper (**5**), but he did not study them carefully and failed to ascribe to them the importance that they really possess. He repeats the statement of his preliminary paper that the chromosomes number constantly five, and thus adds strongly to the case against the individuality of the chromosomes made in the present paper.

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EXPLANATION OF PLATES XVI–XVIII

All the figures were made with a 1.5^{mm} immersion objective and ocular 12 giving a magnification of 2130, excepting *fig. 36*, which was drawn under a 4^{mm} dry lens with a magnification of 400. They were reduced $\frac{1}{3}$ in reproduction, canceling the enlargement due to the camera and rendering them the same size as they were seen in the microscope.

PLATE XVI

FIG. 1.—A vegetative nucleus.

FIGS. 2–4.—Three stages in the formation of the spirem from the same cyst, showing also the asters of the preceding division in process of disintegration.

FIG. 5.—A large nucleus in spirem beside which is a small nucleus, derived by gemmation, still in the vegetative condition; from the same cyst as *figs. 6–9* and *13*.

FIGS. 6–9.—The development of the spindle, from the same cyst.

FIGS. 10–12.—The contraction of the spirem around the equator of the spindle; nuclear membrane with its chromatic granules dissolving; from the same cyst.

FIG. 13.—A young spindle with the chromatin so densely aggregated around the equator that different parts cannot be made out; from the same cyst as *figs. 5–9*.

FIG. 14.—A spindle at metaphase, showing three of the four chromosomes.

FIG. 15.—Chromosomes enlarging as they divide, each of them attached to the pole by heavy fibers.

FIG. 16.—A polar view of metaphase, showing the four chromosomes and the nucleolus.

FIG. 17.—Chromosomes divided and beginning to separate.

FIG. 18.—Anaphase, showing separating chromosomes and the heavy fibers which connect them across the equator of the spindle; from the same cyst as *figs. 15–17*.

FIG. 19.—Anaphase; chromosomes lost in the deeply staining mass at the poles; connecting fibers prominent.

PLATE XVII

FIG. 20.—Anaphase similar to *fig. 19*, but remarkable for the very late persistence of the aster from the previous division.

FIG. 21.—Similar anaphase, with the aster appearing unusually early.

FIG. 22.—Connecting fibers withdrawn.

FIG. 23.—Spindle breaking apart in the middle.

FIG. 24.—Radiations from the chromatic mass just beginning to appear at the lower pole; at the upper pole the focus has shifted out beyond the chromatin.

FIG. 25.—A stage in the development of the aster intermediate between the two poles of the spindle shown in *fig. 24*.

FIG. 26.—Aster strongly developed, while the chromatin remains extremely closely condensed; from the same cyst as *fig. 24*.

FIG. 27.—Chromatin beginning to enlarge to form karyosome.

FIG. 28.—Karyosome rounded off; nuclear vacuole just appearing.

FIG. 29.—Rays of aster beginning to bend around the vacuole to form nuclear membrane; nucleolus and remnant of spindle lying below daughter nucleus.

FIG. 30.—Rays of aster nearly surrounding the nuclear cavity; karyosome passing into spirem of the next mitosis; cf. *fig. 2*.

FIG. 31.—A vegetative nucleus with the old aster lying along side.

PLATE XVIII

FIG. 32.—Spindle of a primary nucleus with halo and disintegrating nucleolus.

FIG. 33.—Two sporangia from a newly segmented summer sorus, showing the character of the mitoses and the independence of the segments.

FIG. 34.—A spindle of one of the last mitoses of the sporangia.

FIG. 35.—*a*, group of spindles formed by the division of a cluster of nuclei formed by amitosis, which lies in the center of a large cyst, the periphery of which contains the spindles of many small nuclei (*b*) derived by gemmation.

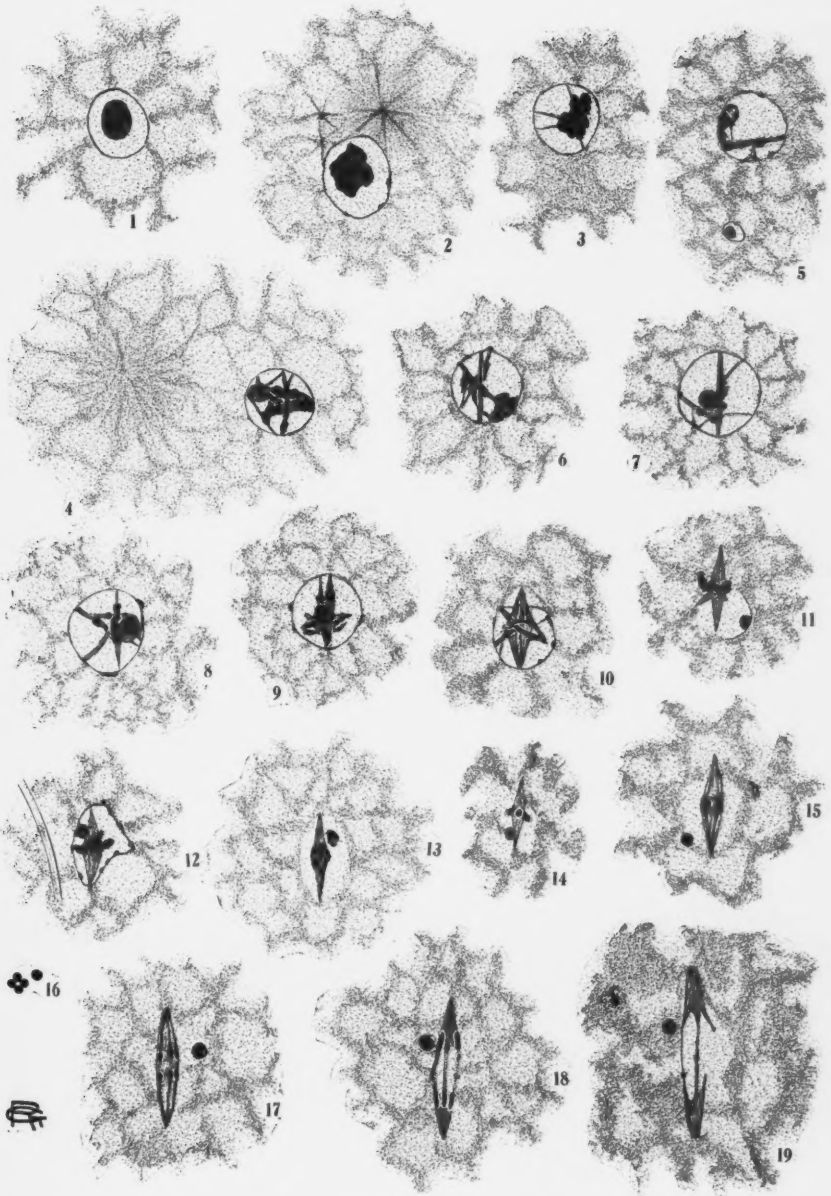
FIG. 36.—Amitosis by constriction in a primary cyst, showing very great differences in chromatin content of daughter nuclei.

FIG. 37.—An amitotic spirem in preparation for nuclear gemmation.

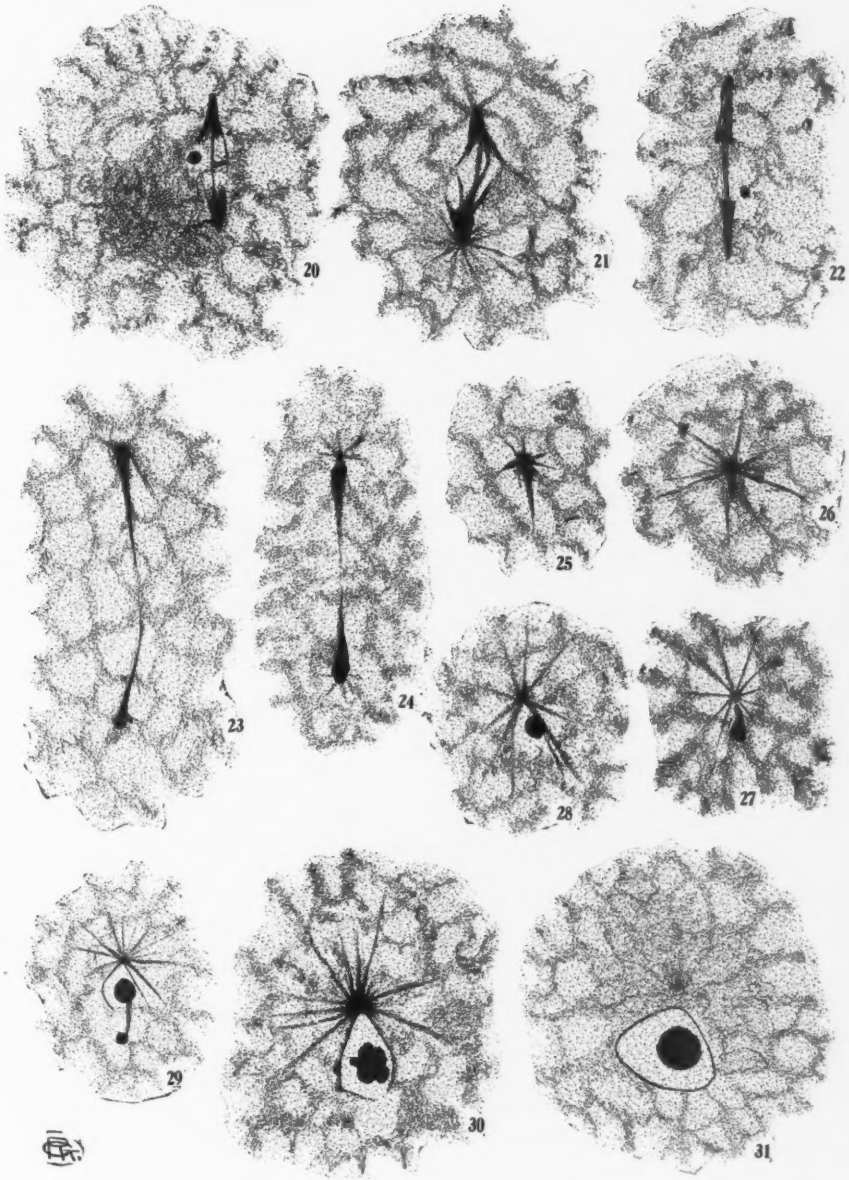
FIGS. 38–40.—Amitosis by constriction and by nuclear gemmation in the same cyst.

FIG. 41.—*a*, two amitotic nuclei; *b*, an adjacent nucleus in spirem, which though undoubtedly amitotic resembles mitotic spirems of *figs. 4, 6; c*, a cluster of amitotic nuclei from the same section.

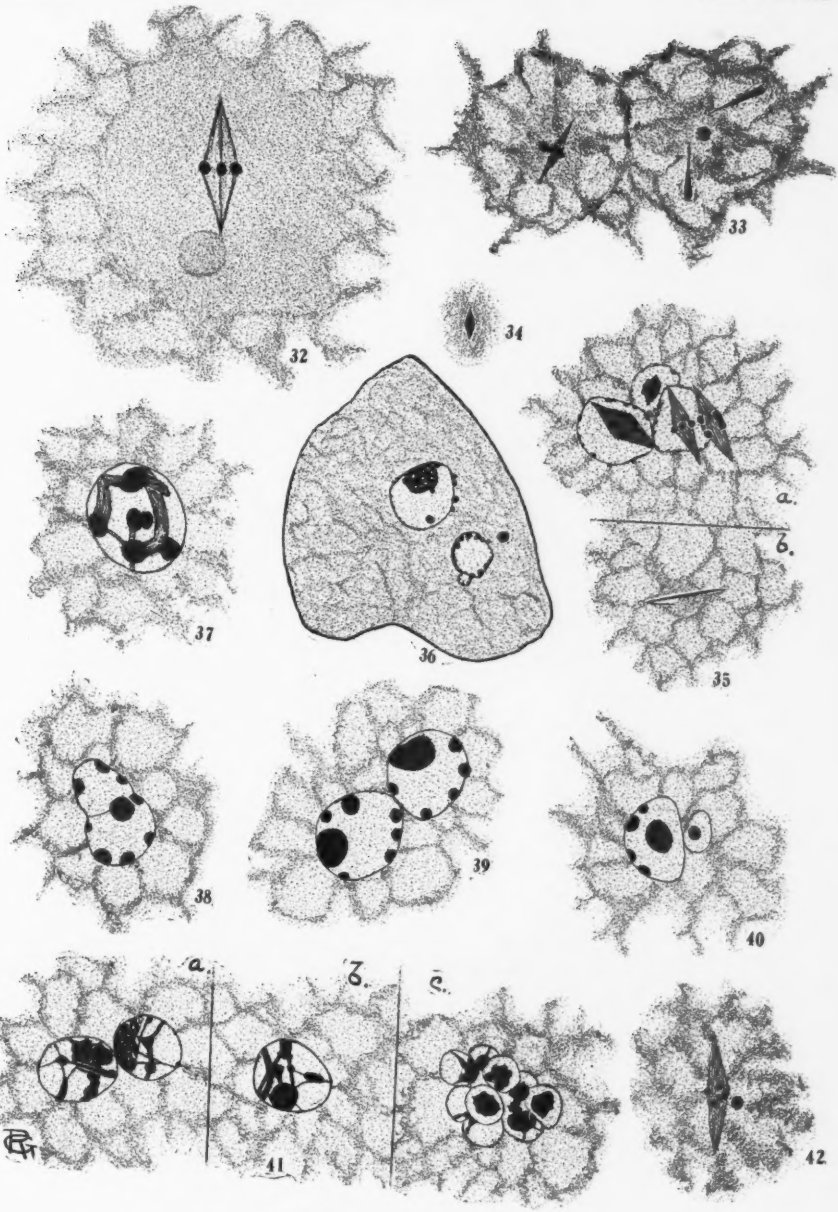
FIG. 42.—A spindle with five chromatin bodies, presumably chromatin granules of the spirem not yet fused into chromosomes; cf. *fig. 11*.



GRIGGS on SYNCHYTRIUM



GRIGGS on SYNCHYTRIUM



GRIGGS on SYNCHYTRIUM

INFLUENCE OF ELECTRICITY ON MICRO-ORGANISMS

GEORGE E. STONE

(WITH TWO FIGURES)

The influence of electricity on the higher plants has been studied for many years, and there is considerable literature pertaining to this subject. The writer has carried on investigations in this line for many years, and some of the results have been published from time to time.

Little or no attention, so far as we know, has been given to the study of the influence of electricity on the growth and multiplication of microorganisms, and it is our purpose to present in this paper the results of some of our investigations of the past two or three years.

Microorganisms are favorable types with which to experiment, since they respond very quickly to stimuli, and, as might be expected, the results are more pronounced than is the case with the higher plants, where growth is relatively slower. The investigations given in this paper were made on microorganisms common to water, milk, and soils, and some experiments were made with yeast. In some instances the natural flora common to water, milk, soils, etc., was used, and in others we experimented with pure cultures.

Influence of electricity on bacteria in water

Our first experiments with the influence of electricity on microorganisms were undertaken in connection with those common to water, and were designed with the object of rendering stagnant water more wholesome by a system of electrical treatment. Our studies had not extended very far, however, before we found that, instead of being decreased by means of this treatment, the bacteria increased enormously, especially when weak currents were employed. In this series we made use of the natural bacterial flora of water, while in others isolated species were experimented with. The experiments were made in glass jars, in some cases those of rectangular form being used, and in others a wide-mouthed bottle. For the purpose of measuring currents we made use of a Weston milliammeter and the

usual bacterial methods were employed throughout these tests. All platings were made in Petri dishes in standard agar-agar, and the usual dilution methods were followed. The agar cultures were incubated at the usual temperatures, but the experiments were conducted at room temperatures in most cases, which ranged from 60° to 70° F.[1]

TABLE I

Showing the influence of electrical stimulation (galvanic currents) on the bacteria in water. First cultures made 24 hours after treatment.

DATE OF MAKING CULTURE	NUMBER OF BACTERIA IN 1^{cc}	
	Normal	Treated
June 19.....	3463	43,642
" 20.....	3435	108,785

The experiment shown in the preceding table was made with two rectangular jars with approximately the following interior dimensions: height 16^{cm}, width 12^{cm}, diameter 4^{cm}. These jars were filled with water obtained from a pond contaminated to a greater or less extent with sewerage. One was a normal or untreated jar, and the other contained electrodes composed of copper and zinc respectively, which were connected with wires and generated a current. The electrodes were of the same diameter as the jar, and one was placed in each end. A jar of this type constitutes a galvanic element (water cell), although the strength of current produced in this case is very weak, averaging about 0.1 milliamperes. Samples of the water were plated in agar-agar 24 hours after treatment. On the second day, however, the experiment was discontinued. The results given in table I show

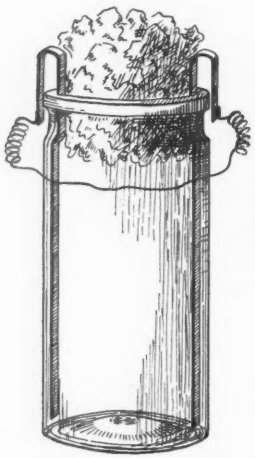


FIG. 1.—Jar, provided with cotton plug and copper and zinc electrodes, used in electrical experiments with milk and water.

[1] In carrying on these experiments the writer is under special obligation to Mr. N. F. MONAHAN, a former assistant in our laboratory, who supervised most of the details of the work.

considerable increase in the number of bacteria in water resulting from electrical stimulation.

The number of organisms in the electrically treated jar increased from about 3000 to 43,000 on the first day, and to 108,000 on the second day.

TABLE II

Showing the influence of electrical stimulation (galvanic currents) on *Pseudomonas radicicola*. First cultures made 24 hours after treatment.

Date of making culture	Number of bacteria in 1cc	
	Normal	Electrical
January 23	6,000	15,000
January 24	50,893	3,178,246
January 27	52,741	4,287,002
January 31	50,217	5,210,112
February 4 ...	50,217	425,000
February 8 ...	42,112	10,200
February 12 ...	41,110	50,000
February 16 ...	35,000	4,000

TABLE III

Showing the influence of electrical stimulation (galvanic currents) on *Bacillus megaterium* DeBary. First cultures made 24 hours after treatment.

Date of making culture	Number of bacteria in 1cc	
	Normal	Electrical
February 25 ...	11,000	243,000
February 28 ...	21,000	3,462,000
March 4.....	25,400	5,600,000
March 8.....	20,000	4,566,400
March 12.....	32,000	7,650,000
March 16.....	10,000	243,000
March 20.....	35,000	500,000
March 24.....	22,000	22,000

The experiments given in the preceding tables were made in wide-mouthed jars approximately 10cm in diameter and 21cm high (*fig. 1*). Those containing the electrically treated water were provided with electrodes made of copper and zinc, which were connected with a wire as in the last experiment. The electrodes were about 4cm wide and long enough to extend over the lip of the bottle. The strength of current developed in this galvanic cell was about 0.3 milliamperes, and it remained very constant throughout the experiments. The

jars in every case were provided with cotton plugs and the whole outfit was sterilized before using. In these experiments pure cultures were used, and the medium, in this case water, was also sterilized before being inoculated. In one series (table II) the jar was inoculated with *Pseudomonas radicicola* (Beyerinck) Moore, from alfalfa; while in the experiments shown in table III the jar was inoculated with *Bacillus megaterium* DeBary, 1^{cc} of a liquid culture medium being used to inoculate the jars in each case. Special care was taken to inoculate the normal and treated jars with the same number of organisms. An examination of the tables II and III will show that there was a marked increase in the number of bacteria during the first few days as a result of electrical stimulation. The maximum in one case was 52,000 for the normal and 5,000,000 for the treated; in another case 32,000 for the normal and 7,000,000 for the treated. It will be noted that the subsequent decrease in the number of the organisms was very marked in the electrically stimulated cultures, a feature due to the accumulation of zinc oxid in the jar, which is always present as a white precipitate in galvanic cells of this type. The presence of zinc oxid in water formed by the action of even comparatively weak currents is toxic to bacteria, and the same toxic effect is well illustrated in galvanotropic experiments with roots. Some of our experiments which were made in much smaller jars failed entirely, as the smaller volume of water employed became concentrated so quickly with this substance that a toxic effect on the organism occurred very shortly after inoculation. Some of the precipitate obtained was dried and dissolved in flasks containing sterilized water. The jars were then inoculated with *Bacillus megaterium* DeBary, with the result that very little increase in the number of bacteria occurred where a 2 per cent. solution of this prepared precipitate of zinc oxid was used, and a 10 per cent. solution apparently killed all bacteria. In both of the experiments enumerated there occurred a slight falling-off in the number of organisms in the normal or untreated cultures. This is of common occurrence, however, in standing water, or even in soils under certain conditions.

The strength of current developed in these experiments (0.1 and 0.3 milliampere) was very constant, and from the results obtained it is evident that it acted as a marked stimulus. A large series of

experiments made by us on the higher plants has shown that this current strength is very close to the optimum, and in all probability the optimum current strength for bacteria would differ little if any from that of the higher plants. We have employed this method of electrically stimulating bacteria and have enormously increased the number of organisms in cultures containing the legume Pseudomonas, which is used in inoculating soils.

Some experiments were also carried on at the same time relative to the influence which electrical stimulation might have upon nitrogen fixation, but the results are incomplete and will not be given in this paper.

The influence of electricity on bacteria in milk

The purpose of our experiments in this series was similar to that in the experiments made with water; that is, to determine the effect of electrical stimulation on the microorganisms in milk. Our object, however, was not only to ascertain the effects of optimum currents, or at least those approximating the optimum on the bacteria of milk, but to observe the effects of strong electrical charges.

Milk constitutes an excellent medium for the multiplication of bacteria and is well suited in some respects to experiments of this nature. The experiments given in tables IV and V were conducted similarly to the ones shown in the preceding series; that is, the bacteria were stimulated by galvanic currents and the same size culture jars were used (*fig. 1*). About 1.5 pints of unsterilized milk were placed in each jar and a milliammeter indicated the strength of current to be approximately 0.3 milliampere in the electrically treated samples. The jars were provided with cotton plugs and were sterilized before being filled with milk. The usual dilution methods were followed and the standard agar-agar was used for plate cultures. In practically all instances the counts are averages of three and four plates. Platings were made of the milk at the beginning of the experiment, that is, before being electrically stimulated; therefore these counts, which are averages, answer for both the treated and untreated cultures.

The results of electrical stimulation on bacteria in milk are shown in the experiments given in tables IV and V, but since milk sours and curdles badly in a few days it was necessary to limit the duration

of the experiments. In the normal or untreated samples the increase in the number of organisms in one experiment was from 143,000 to 6,000,000; while in the treated samples the number reached 94,000,000. In the experiment shown in table V the normal increased from 118,000 to 4,000,000; while the electrically treated reached 83,000,000. The results are more striking than those obtained by the treatment of water, as might be expected, since there was more food available for the use of the organisms in the latter series.

TABLE IV

Showing the influence of electricity (galvanic currents) on the bacteria in milk

Date of making culture	Number of bacteria in 1cc	
	Normal	Electrical
May 16, 2 P. M.	143,395	143,395
May 17, 9 A. M.	809,112	3,874,421
May 17, 5 P. M.	1,470,441	86,592,600
May 18, 9 A. M.	6,082,542*	94,851,806*

* Milk sour.

TABLE V

Showing the influence of electricity (galvanic currents) on the bacteria of milk

Date of making culture	Number of bacteria in 1cc	
	Normal	Electrical
May 17, 10 A. M.	118,542	118,542
May 17, 5 P. M.	678,333	1,848,806
May 18, 10 A. M.	1,026,533	41,778,766
May 18, 5 P. M.	4,591,500*	83,363,866*

* Milk sour.

Another series of experiments was undertaken to demonstrate the effects of static electricity on bacteria in milk. For this purpose we employed a static machine of the Töpler-Holtz type, which was designed for X-ray work and is capable of producing a spark six inches or more in length. The method of plating, etc., was the same as has been previously described, and the culture jars were of the same type, except that the electrically stimulated jars were covered with tinfoil in the same way as a Leyden jar. The copper and zinc plates were dispensed with, of course, and the milk was charged direct from the static machine. In this series three jars were used:

one normal, one treated with positive, and one with negative charges. The milk in the electrically treated jars was charged with sparks from a static machine in the same way that a Leyden jar is charged, an electroscope being used to determine the nature of the charge. In the milk experiments which follow it should be pointed out that the charges varied considerably.

TABLE VI

Showing the influence of static electricity (positive and negative charges) upon the bacteria in milk. Electrical jars charged with one spark each June 11.

Date of making culture	Number of bacteria in 1cc		
	Normal	Electrical (positive charge)	Electrical (negative charge)
June 11	8,342	8,342	8,342
June 12	60,000	196,300	210,000
June 13	568,000	1,240,000	1,367,500
June 14	1,213,000	16,432,500	19,374,600
June 15	9,876,400	70,500,000	79,600,000
June 16	27,432,000	153,461,000	131,540,000
June 17	190,500,000*	267,000,000*	233,330,000*

* Milk curdled.

TABLE VII

Showing the influence of static electricity (positive and negative charges) upon the bacteria in milk. Electrical jars charged with 10 sparks each June 2.

Date of making culture	Number of bacteria in 1cc		
	Normal	Electrical (positive charge)	Electrical (negative charge)
June 2, 10 A. M	565,000	565,000	565,000
June 2, 5 P. M.	1,173,000	597,666	624,333
June 3, 10 A. M.	19,057,000	23,443,666	18,088,333
June 3, 5 P. M.	107,440,000	151,516,000	125,502,000
June 4, 10 A. M.	201,413,333*	287,380,000*	212,816,666*

* Milk sour.

In the experiment shown in table VI one large spark was given each electrically treated jar, one being given a positive and the other a negative charge, and in the one shown in table VII the number of sparks was increased to 10. The electrical treatment, the results of which are shown in table VI, where the milk was charged with a single spark and cultures made every 24 hours, gave rise to a decided acceleration. This acceleration was perceptible at the time of the

first plating and continued throughout the experiments, which lasted six days.

The results shown in table VII indicate that the treatment given caused less acceleration. In this case platings were made at shorter intervals. The first plating was made seven hours after stimulating; at this time scarcely any acceleration was shown, which indicated the possibility of the death of the organisms by treatment, while during the same period the normal nearly doubled in the number of bacteria. The later platings, however, made at 24, 31, and 48 hours respectively after stimulation, showed a greater increase than that given by the normal, but the ten charges given in the latter experiment were evidently too strong to obtain the optimum results. In both the experiments enumerated (tables VI and VII) the milk was kept on ice.

TABLE VIII

Showing the influence of static electricity (positive and negative charges) upon the bacteria in milk. Electrical jars charged with 100 sparks each.

DATE OF MAKING CULTURE	NUMBER OF BACTERIA IN 1CC		
	Normal	Electrical (positive charge)	Electrical (negative charge)
May 31, 10 A. M.....	528,000	528,000	528,000
May 31, 5 P. M.....	1,546,000	568,333	601,100
June 1, 10 A. M.....	24,885,344	1,323,333	916,666
June 1, 5 P. M.....	164,033,000	2,144,600	2,133,460
June 2, 10 A. M.....	225,103,632	15,068,333	13,631,090
June 2, 5 P. M.....	200,500,000	84,654,000	45,612,000
June 3, 10 A. M.....	149,930,000	102,032,420	83,533,220

In another experiment (table VIII) the number of sparks was increased to 100 and the electrically treated jars were charged at 10 A. M. and 5 P. M. each day for a period of three days, with the result that a decided inhibitory effect was noticed after the first treatment, followed by a less increase for the succeeding periods than that given by the normal, and on the third day, when the experiment was discontinued, the electrically treated milk showed a smaller number of bacteria than the normal, thus showing that electrical charges were too strong for the maximum development of the organisms.

A much greater inhibitory effect is shown in table IX, where 100 sparks were applied at more frequent intervals—10 A. M., 1 P. M.,

and 5 P. M. respectively for two days. At the close of the experiment there was little or no difference between the normal and the treated samples. The subsequent charges, however, failed to prevent an increase in the number of bacteria.

TABLE IX

Showing the influence of electricity (positive and negative charges) on the bacteria in milk. Electrical jars charged with 100 sparks each 10 A. M. and 1 P. M. and 5 P. M. each day.

Date of making culture	Number of bacteria in 1cc		
	Normal	Electrical (positive charge)	Electrical (negative charge)
July 3, 10 A. M.....	5,580	5,580	5,580
July 3, 1 P. M.....	21,440	381	404
July 3, 5 P. M.....	78,533	380	487
July 4, 10 A. M.....	268,500	5,664	10,367
July 4, 1 P. M.....	863,830	10,806	26,990
July 5, 10 A. M.....	17,800,000*	19,180,000*	19,910,000*

* Milk sour.

TABLE X

Showing the influence of electricity (positive and negative charges) on the bacteria in milk. Electrical jars charged with 50–100 sparks each every hour (10 A. M. to 5 P. M. inclusive).

Date of making culture	Number of bacteria in 1cc		
	Normal	Positive	Negative
July 6, 10 A. M....	219,250	219,250	219,250
July 6, 3 P. M....	1,000,000	481	266
July 6, 5:30 P. M....	10,655,000*	11,522*	935*

* Sour.

As it was one of the objects of these experiments with milk to determine the maximum stimulus, the charges were increased. For this reason the experiment shown in table X was made, where an even more marked falling-off in the number of organisms is shown. The experiment lasted only a few hours, and was not continued on account of the souring of the milk.

The hourly charges of 50 to 100 large sparks, however, did not entirely prevent the organisms from developing, but the falling-off from over 200,000 to a few hundred in 1 cc was significant.

In another experiment, where only one series of heavy charges was given, the number of organisms decreased from 31,000 to 35 one-half hour after treatment; while on the third day the number in the normal was 29,000,000 against 1,000,000 of the treated. Further experiments of a similar nature were made, but in no case were we able to prevent the subsequent appearance of organisms.

The immediate falling-off in the number of bacteria when strong and frequent charges from a static machine were employed would point to the conclusion that the electrical treatment destroyed the bacteria, but what effect it may have had on the spores was not determined. The ultimate increase in the number of organisms in every case after treatment, where heavy charges were used, is also significant, and may be explained on the supposition that strong electrical charges did not affect the spores; in other words, the immediate falling-off in numbers resulting from excessive stimulation may be due to the destruction of the vegetative forms, and those which did appear in the agar cultures may have developed entirely from spores which were possibly not affected detrimentally by treatment. Furthermore, there is a possibility that the strong static charges might induce a tendency in the organisms to spore formation, and the spores, not being affected by the heavy charging, would germinate in the agar cultures.

In the experiment shown in table IX, where a considerable falling-off in the number of organisms took place at first, which was followed by subsequent increase, there was a long period in the night when no stimulation was applied, and spore formation may have taken place; but in the experiment shown in table X the period elapsing between treatments was only one hour, yet the number of bacteria in one instance increased very perceptibly; namely, in the positively charged culture. On the other hand, if the case is one of inhibition or suppression of the vital processes only, we should expect this effect to be lost in the period elapsing between plating and the counting of the colonies. The possibility of accommodation or adaptation of the organisms to intense stimulation is also not out of the question, as this frequently occurs. The roots of certain plants, for example, can grow and develop in water saturated with poisonous gases if given an opportunity to adapt themselves to these extreme conditions,

whereas these plants, if developed normally and placed under these conditions, would suddenly collapse. Our experiments do not furnish sufficient data to determine which of these views is correct. The maximum stimulation would undoubtedly be different for spores than for bacteria existing in the vegetative stage, as it is well known that those in the vegetative stage succumb more readily to heat than those in the spore form. The effects of electricity on spores can best be determined by experiments with pure cultures possessing certain characteristics, rather than those of heterogeneous types such as characterized the flora of the milk with which we experimented.

In our experiments with milk and water, where galvanic currents were employed, the stimulus was constant, whereas when only one shock or a series of shocks was given the organism from a static machine the charges soon disappeared, although the effects of electrical stimuli of brief duration give rise to decided reactions.

It should be pointed out that an increase of even twenty fold in the number of organisms in a given solution at the outset would make a vast difference in the number a few days later, even if the same subsequent rate of increase followed in both the normal and treated cultures. The amount of available food supply in a solution, however, is limited, and in the end there is often little difference in the number of organisms present in any treated or untreated series.

Undoubtedly the use of strong electrical currents is capable of destroying bacteria and preventing milk from deteriorating, although other methods of electrical treatment would probably prove more satisfactory than those which we employed. In some tests made of electrically treated milk we found that souring was delayed. It is well to note in passing that there appears to be a difference in the effects of the positive and negative charges; for example, if a comparison is made of the averages of the last counts in tables VI to X inclusive, it will be found that the milk treated with positive charges gave a larger count than that treated with negative charges. The number of bacteria shown in the positively charged jars was 135,000,000, while that of the negatively charged was 109,918,164. This is what might be expected, since the writer has previously demonstrated in a large series of experiments on the growth of seedlings that positive charges stimulated both roots and stems more than the negative charges.

Feeble electrical currents and small static charges act as stimuli to bacteria in milk, increasing their numbers very perceptibly, and under certain conditions there may be after all some foundation for the old belief that milk sours more quickly during thunderstorms than at other times. Notwithstanding the fact that there may be other conditions during thunderstorms, such as warm and sultry weather, which may accelerate bacterial action, it is not difficult to imagine conditions under which milk might be stored which would subject it to electrical stimulation, thus increasing the number of bacteria and incidentally hastening souring.

The influence of electricity on bacteria in soils

Only a limited number of experiments was made by us relative to the influence of electricity on bacteria in soils. Careful bacterial analyses of soils are tedious operations and the methods followed in these analyses were similar to those recommended and used by CHESTER.[2] In all cases the counts were made on one gram of air-dried soil. In these experiments we made use of wooden boxes 8×8×8 inches, inside measurements, which were filled with fairly good loam free from coarse material. The soil used was more or less compact, very fine sand (0.01^{mm}–0.005) predominating. It might be expected that the texture of the soil, as determined by the size of the particles, the amount of organic matter, and plant food, would exert an important influence on the bacterial flora, and the rather fine texture of the soil which we selected for this experiment would give results different from those that would be obtained from a looser soil containing larger particles and having more air space, or one containing a larger amount of organic matter.

In the box electrically treated were placed copper and zinc electrodes, each being 8×8 inches in size, and to these were soldered copper wires which were connected, thus forming with the soil a galvanic cell which furnishes a small current approximating the optimum. The percentage of water in the soil in each box was accurately determined at the beginning of the experiment, and this same percentage was maintained throughout by adding sterilized distilled water. The experiments were carried on in the laboratory under conditions as

[2] CHESTER, F. D., Delaware Agric. Exper. Sta. Bull. 65 : 61–65. 1904.

nearly identical as it was possible to make them, the soil being exposed to the air continually. The cultures were plated in agar-agar and the usual dilution methods were followed.

TABLE XI

Showing the results of electrical stimulation (galvanic currents) on the bacteria in soil.

	Date of sampling	Number of bacteria in 1gm	
		Normal	Electrical
Experiment I.....	July 13	33,470,000	37,930,000
	July 29	28,777,000	32,863,000
	August 11	19,294,000	35,000,000
Experiment II.....	September 14	38,047,000	37,670,000
	October 16	18,720,000	26,384,000

Two experiments are given in this table, the first date of sampling corresponding with the beginning of the experiment. No attempt was made to disturb or cultivate the soil in the boxes, and the surface became more or less compacted by constant watering, which no doubt accounts for the general falling-off in the number of bacteria in all cases. It will be noted that no increase is shown in the number of bacteria in either the treated or untreated soils, although the extent of falling-off in both experiments is less in the electrically treated boxes. Similar experiments were made with soil in the same boxes with static electricity. The boxes in this case were arranged as follows: one box was left normal as before, and the other had 12 wires extending into the soil leading to a metal bulb which was given 100 sparks from a Töpler-Holtz machine once a week. In this case the first samples were taken a few days after electrical treatment; otherwise similar methods were employed as in the preceding series.

In this case there did not occur the same falling-off or decrease in the number of the organisms in either the treated or untreated cultures at the time of the subsequent platings, as in the preceding series. On the other hand, the treated soil in this experiment showed a continual increase in the number of bacteria present at the time of the different platings. This was apparently due to the frequent stirring or cultivation of the soil. The number of organisms, however, showed only a slight increase at the time of the first platings, whereas the

electrically treated increased from 4,000,000 to 27,000,000. The soil in this case was freshly prepared and carefully mixed and contained more organic matter and plant food than the former; neither was there the same tendency for the soil to become badly compacted as in the preceding series.

TABLE XII

Showing the results of electrical stimulation on the bacteria in soil (static electricity).

Date of sampling	Number of bacteria in 1gm	
	Normal	Electrical
July 21.....	1,097,290	4,506,700
July 31.....	960,000	15,208,000
August 7.....	1,960,780	27,756,000

The electrical experiments with soil were not continued, since the details associated with soil bacteriological analyses are laborious. The results of electrical stimulation of soil organisms are not so pronounced as in the case of the water and milk experiments, but the effects are clearly shown in table XII.

Our numerous experiments in growing plants in electrically stimulated soils have demonstrated that considerable acceleration in germination and growth follow when currents of optimum intensity (0.1–0.6 milliampere) are employed, and all the forms of plant life are undoubtedly stimulated in a similar way.

Influence of electrical stimulation on yeast

Some experiments were made in our laboratory for the purpose of observing the effects of electrical stimulation on yeast, in which we endeavored to determine the relative activity of the normal and electrically treated organisms by the amount of CO_2 given off.

As in the preceding series, we used galvanic currents obtained by the use of copper and zinc electrodes and also static charges from a Töpler-Holtz machine. Small bottles were used in the experiments, ranging from 2 to 4^{cm} in diameter and 12 to 16^{cm} high. The electrodes were 2^{cm} in diameter and were placed in the bottle containing the yeast and culture media. In the experiment with static electricity we made use of simple Leyden jars corresponding to those described

in the preceding series. In all instances corresponding jars were employed in the normal and electrically treated yeast, and the conditions were identical so far as they could be made in every way in all cases. From 0.5 to 5^{gm} or more of yeast were placed in each jar in the various experiments, and either a solution of molasses (about 5 per cent.) or a standard nutrient solution was used. The nutrient solution was made up as follows:

	Grams
Calcium nitrate	6.0
Potassium nitrate	1.5
Magnesium sulfate	1.5
Neutral potassium phosphate	1.5
Sodium chlorid	1.5
Cane sugar	125.0
Distilled water	2500.0

This solution was not chosen on account of its being the best adapted for this purpose, but it proved to be satisfactory.

The yeast used in these experiments was from the ordinary commercial yeast cakes, which were cut into cubes and carefully weighed. The yeast was then put into mortars containing the nutrient solution and the cells carefully separated by repeated stirring. After the yeast cells were well separated they were placed in their respective bottles. The mortar was thoroughly rinsed and care was taken to have the same amount of yeast in each. The bottles containing the yeast were completely filled and connected by means of glass tubes to graduated cylinders or burettes containing water, and as CO_2 was given off, the displacing of water was noted at intervals and recorded. The following table gives the results of some experiments with yeast.

The experiments (see table XIII) had a duration of 1.5 hours to 4 days, and in all instances the amount of CO_2 given off was greater in the electrically treated than in the normal or those not stimulated. The experiments were conducted on different days and at different temperatures, most of them being at room temperatures, and as there was no heat on in the laboratory at the season of the year in which many of these experiments were conducted, the temperature was naturally somewhat variable. In *a*, *b*, *c*, *g*, and *h*, however, the bottles containing the yeast were in water baths and were kept under conditions nearer to the optimum for yeast (32–38° C.), hence the per-

centage of CO_2 given off in these was greater for a given period than in some of the others. All of the other experiments were run at room temperatures, which in some instances were fairly good, and in others the room was too cool to expect much activity on the part of the yeast. The small amount of CO_2 given off in some instances is therefore due to the temperature conditions under which the experiment was made, and moreover, since these experiments in some instances lasted four days, there was more or less absorption of CO_2 by water in the graduated cylinders.

TABLE XIII

Showing the influence of electricity on yeast

EXPERIMENT	NATURE OF ELECTRICAL STIMULUS	DURATION OF EXPERIMENT	NUMBER OF CC OF CO_2 GIVEN OFF IN	
			Normal	Electrical
a	galvanic	2 hours	174	212
b	galvanic	3 days	752	838
c	galvanic	2 hours	189	232
d	galvanic	3 days	540	700
e	galvanic	4 days	20	296
f	galvanic	4 days	50	1200
g	static*	1.5 hours	112	129
h	static	2 hours	74	102
i	static	7 hours	82	120
j	static	3 days	922	1035
k	static	2 days	300	575
l	static	4 days	15	140

* *g* was given 8 sparks; *j* and *l*, 5 each; *k*, 1 spark; *h*, 2; and *i*, 3.

The absorption of CO_2 by water was noticeable when the yeast cultures were left over night, especially when the room temperature was low and the yeast not active. The absorption of water, however, was apparently the same in both the treated and untreated series and did not affect the relative results. In some cases, therefore, a larger amount of CO_2 was given off than is shown by the records in the tables.

Frequent observations and readings were made of the amount of CO_2 given off, but to make the tables brief we have given only the final readings.

The effects of electrical stimulation seem to be more pronounced in the lower temperature experiments than in those where there was a high temperature. In some instances the untreated cultures gave

off very little gas, while the treated, under the same temperature conditions, gave off considerable. The electrical stimulation, under these conditions, appeared to act very much as yeast would if subject to an increase in temperature.

In those experiments where the carbon dioxid gas readings were made at short and regular intervals, we were able to observe the effects of the stimulation on the organisms at different periods.

In some experiments the observations were made every five minutes from the start, and after a number of observations were made and recorded, the stimulus was applied. The results obtained in one of the experiments, in which five-minute observations were made, are given in the curve shown in *fig. 2*.[3] This curve is based on the increased amount of carbon dioxid given off by the normal or untreated; in other words, the amount of CO_2 generated by the normal in this case would be represented by the base line, or it would be equivalent to zero. The treated yeast was given two very small sparks from a static machine at 1:50 P. M., or one-half hour after the experiment was started. A temperature of 30–35° C was maintained during the experiment.

The relative amount of CO_2 given off by the electrically treated and untreated yeast before the stimulation was applied showed quite a uniform activity on the part of the yeast. Following the latent period, which is usually of 15 to 25 minutes' duration, the results of the treatment were manifested in considerable acceleration in gas production. This reached its maximum effect at 3:20 P. M., or 1.5 hours after the stimulation had been applied. Another stimulus consisting of two minute sparks from a Töpler-Holtz machine was applied at 3:45 P. M. and produced a brief reaction, as shown by the curve. The experiment, however, was discontinued at 4:20 P. M.

In the experiments with yeast there was considerable variation in the number and size of the sparks applied to the treated jars, and there appears to be an indication, from the results obtained, that in some instances the electrical charge was too severe. This was noticed in the short-interval readings immediately following the stimulation, and in such cases the maximum acceleration period was more remote

[3] We are indebted to Mr. G. H. CHAPMAN, assistant in the laboratory, for supervising this experiment, as well as one other in this series.

from the stimulation period than in this case, where very weak charges were used. A charge of one or two minute sparks from a Leyden jar seemed to cause the most active response on the part of the yeast.

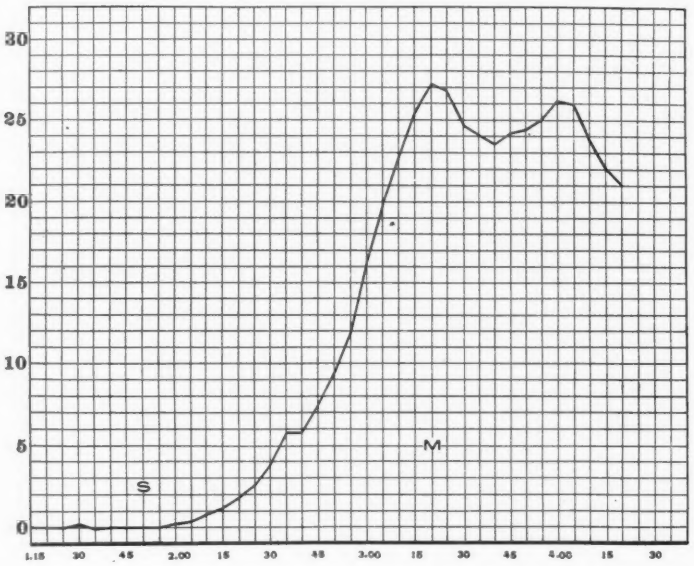


FIG. 2.—Curve representing the amount of CO_2 given off by electrically stimulated yeast; the horizontal lines represent the amount of carbon dioxid in cubic centimeters; the vertical lines five-minute periods of observation; *S*, time stimulation was applied (1:50 P. M.); *M*, maximum effect of stimulus.

Discussion of results

In considering the results presented here it should be pointed out that little attempt was made to ascertain the strength of the current necessary to produce the best results, and the static charges employed differed in number and intensity. We have already demonstrated in a large series of experiments[4] with the higher plants that the optimum current for germination of seeds and growth of seedlings is not far from 0.1 milliampere, and in our study of the effect of static

[4] STONE, G. E., The influence of current electricity on plant growth. Ann. Rep. Hatch Exper. Sta. Mass. Agric. Coll. **16**: 13–31.

charges on the germination of seeds and growth of seedlings we have observed that a very few minute sparks from a static machine caused the most marked stimulation.

In regard to the influence of atmospheric electrical potential on growth, MONAHAN[5] found that when the air in a glass case is charged to a potential of about fifty volts, better results were obtained than when a higher potential was used. We endeavored, therefore, in these experiments to make use of current strengths approximating the optimum, or that strength which gave the best results in our previous investigations with various organisms, except in those cases where strong static charges were given to milk for the purpose of ascertaining the degree of stimulation which would kill the organisms.

The results obtained from these researches suggest many lines of work which might be followed, but we are obliged to discontinue them for the present. Electricity undoubtedly, in one way or another, plays a very important rôle in plant life. Seed germination and growth of seedlings are greatly accelerated by feeble currents, but, unlike amides and enzymes, they are incapable of affecting the germinating capacity or of regenerating, as it were, the life in the seeds. The roots of the higher plants exist in a medium which is charged negatively, and the electrical potential of the air is often quite high within the limits of large trees. The electrical potential under the foliage of a tree is less than that at corresponding heights in the free atmosphere.[6] When, however, there is no foliage, the electrical potential under the branches of trees corresponds to that of the free air at equal heights, and there is reason to believe that the apices of leaves are merely so many points for the gathering and discharge of electricity. Minute currents of electricity exist in plants, and it is known that during certain periods trees discharge sparks from the apices of the leaves, and trees may tend to equalize differences in potential existing between the earth and air. Rain drops in falling become electrically charged, and as they gather microorganisms in their descent through the air, these also probably become affected. The remarkable influence of rain upon

[5] MONAHAN, N. F., The influence of atmospheric electrical potential on plants. Ann. Rep. Hatch Exper. Sta. Mass. Agric. Coll. **16**:31–37.

[6] STONE, G. E., AND MONAHAN, N. F., Ann. Rep. Hatch Exper. Sta. Mass. Agric. Coll. **17**:13–31.

vegetation cannot be satisfactorily explained, in our opinion, by chemical analysis or by the various other conditions which prevail, and the idea that electrical stimulation plays an important rôle here is not an improbable one. It is also not unlikely that during thunderstorms the bacteria in milk are affected, although two series of experiments made by us in exposing milk in sterilized metal vessels at different elevations, where the electrical potential showed considerable variation, were by no means conclusive.

The effects of electrical stimulation on plant growth resemble more nearly those produced by heat, that is, in the tendency of the plant to assume a rather spindling growth; but this similarity in the method of reacting does not necessarily prove that electricity and heat are identical, since spindling growths in plants occur from other causes. The effects of electrical stimulation do not resemble those induced by light, since light inhibits growth; on the other hand, they more closely resemble the effects induced by lack of light (partial etiolation) and other forms of stimulation which may be produced by various agencies. Electricity stimulates seeds very perceptibly, causing an acceleration in growth, and probably has the same effect on spores, and in this way the number of bacteria in solutions might be increased. The process of cell division of bacteria and the budding of yeast are undoubtedly stimulated by electricity, which would result in an increase in the number of organisms and an acceleration of the metabolic process.

The effects of electrical stimulation, like other types of stimuli, are manifested shortly after application. With a current of optimum intensity a latent period occurs when no effect is discernible, and this is followed by an acceleration in growth and development. The nature of the response is dependent upon the intensity of the stimulus as well as upon its duration; therefore to determine the period of duration of any particular response or its maximum period, the intensity and duration of the stimulus must be taken into consideration. Since the intensity and duration of the stimulus employed in these experiments differed materially, the response periods would also vary accordingly. As regards the manner in which electricity stimulates organisms, little can be said at the present time, and the problem is as difficult of solution as the manner in which light, etc., affect the organisms.

Many theories, however, in regard to the cause of the stimulating effect of electricity on plant growth have been advanced, some of which are hardly worthy of consideration, since they fail to meet the requirements of experiments, and we will not enter into a discussion of them here.

Electricity, like other forms of stimulation, such as light, heat, etc., undoubtedly affects the protoplasm of the plant, which causes certain metabolic processes to become active and accelerated growth results. In plants showing circulation and rotation of protoplasm, e. g., Chara, Nitella, etc., feeble electrical currents induce a more rapid streaming of the protoplasm, which is undoubtedly associated with greater metabolic activity, and it is not at all unlikely that changes of a similar nature take place in other organisms when subject to feeble electrical currents.

AMHERST, MASS.

CYTOLOGY OF CUTLERIA AND AGLAOZONIA

A PRELIMINARY PAPER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **129**

SHIGÉO YAMANOUCHI

This preliminary note gives a brief account of my cytological studies of *Cutleria multifida* Grev. and *Aglaozonia reptans* Kütz. The material was collected last winter and spring at Naples, where I occupied a table of the Carnegie Institution at the Zoological Station. The work was begun at Naples and was continued at the University of Chicago under the direction of Professor JOHN M. COULTER and Professor CHARLES J. CHAMBERLAIN, to whom I wish to acknowledge my great indebtedness for their suggestions and criticisms. Many points of cytological interest and importance, as well as the discussion of literature, will be presented in the full account to be published later.

Cutleria multifida

GAMETOGENESIS

Cutleria multifida is generally dioecious. The young thallus, $1–3^{mm}$ long and narrowly fan-shaped, presents no features to distinguish between the male and female plants. When the thallus has reached the stage for the formation of reproductive organs, the habit of the male plant is occasionally different from that of the female; but an extensive comparative study of the forms suggests that there is great variability in habit, so that it seems impossible to distinguish the two sexual individuals by any morphological character except that they bear as a rule exclusively either male or female organs.

VEGETATIVE MITOSIS IN BOTH MALE AND FEMALE INDIVIDUALS.—Both male and female plants, in good condition, always have a hairy growth at the tips of the multifid filaments of the thallus. Any part of the frond in vigorous growth is favorable for the study of vegetative mitosis, but details are more easily followed in the terminal hairs and in the superficial layer of the entire frond.

The cells in these regions are full of plastids, with usually a single nucleus in the center. The nucleus in the resting state is very small, generally about the size of the plastids or sometimes a little smaller. The network is so finely built that it is hard to recognize much chromatin in it. Neither centrosomes nor central bodies with or without radiations seem to be present.

In early prophase the nucleus increases in size until it is twice the diameter of the resting nucleus and occupies a greater part of the cell, pushing aside the numerous plastids toward the periphery. During the growth of the nucleus, there appear just inside of the membrane chromatin knots which are evidently worked out from the chromatin network by the rearrangement of the material. These chromatin knots, which are of course in continuation with less deeply stained chromatin fibrils, are variable in number at first, but gradually there appears a certain number of chromatin knots that are afterward detached from the chromatin fibrils and become chromosomes, 24 in number. The chromosomes after segmentation gradually assume a slightly elongated rod-form and become arranged at the equatorial plate.

A little before the equatorial plate stage, two kinoplasmic accumulations arise from the cytoplasm surrounding the nuclear membrane at two poles. A well-marked central body in the kinoplasmic mass occurs only at late metaphase. The chromosomes split longitudinally and half of each chromosome proceeds to each pole. During this entire process the spindle is intranuclear. At telophase the nuclear membrane disappears and the two sets of daughter chromosomes, in a state of close aggregation, are now surrounded by cytoplasm, and the formation of the nuclear membrane follows.

When the daughter nuclei are organized, the central spindle disappears completely. The cytoplasm lying between the two nuclei begins gradually to assume a coarse, irregular, alveolar structure, and the walls of the alveoli, probably after a change in their material, form a new cell plate.

Thus vegetative mitosis agrees in its essentials in both male and female plants.

FORMATION OF MALE GAMETES.—When the male plant is young, the surface of the thallus bears tufts of hairs here and there in some-

what regularly scattered spots. Later, with or without association of hairs, there are produced short filaments which afterward bear male gametangia. Both the filaments and the hairs arise from superficial cells of the thallus.

One of the superficial cells commences to grow more vigorously than the rest and a typical nuclear division takes place. Two or more subsequent divisions result in a short filament of three or more cells, the terminal one of which is destined to be a male gametangium initial, whose nucleus becomes considerably larger than is common in cases of vegetative mitosis. The mitoses which take place up to the formation of the male gametangium initial are typical and the number of chromosomes is 24.

The details of nuclear division are much more easily and distinctly followed in the gametangia. During the prophase of the first division in the gametangium initial, even before the segmentation of chromosomes, the nucleus is marked by two distinct kinoplasmic accumulations at the poles, and their position indicates the axis of the division. The formation of the cell plate between two daughter nuclei is sometimes much more delayed than in cases of vegetative mitosis.

Following the first division in the gametangium there are several cell divisions, the walls being somewhat perpendicular to one another; as a result there is formed the well-known male gametangium of Cutleria, composed of a great number (sometimes as many as 200) of mother cells, regularly arranged in vertical and horizontal tiers. During all of these successive divisions 24 chromosomes appear.

The nucleus, cytoplasm, and plastids in the mother cell undergo a certain peculiar change, and the whole contents of the mother cell enter into the formation of a male gamete. After the maturity of the gamete a tiny hole is developed in the peripheral wall of the mother cell, through which the gamete escapes.

FORMATION OF FEMALE GAMETES.—The formation of tufts of hairs and of filaments bearing female gametangia is similar to that already described for the male plant. The structure of the cells of the superficial layer is apparently like that of the male plant, and so far as the development of hairs and the behavior of nuclei are concerned, there seems to be no distinction; but the difference is remarkable when the superficial cell which is destined to form a female gametangium begins

to grow. The growth usually proceeds until the cell becomes two or more times as large as the corresponding cell of the male plant. Curiously enough the nuclear growth does not keep pace with that of the cell; in other words, the nucleus in the superficial cell at the time of division has almost the same dimensions as in the male plant, and this equality persists up to the formation of mother cells.

The first division of the superficial cell is followed by two or more divisions, which result in a short filament whose terminal cell becomes a female gametangium. A number of divisions in the gametangium produces eventually a structure composed of regularly arranged mother cells.

The nucleus and cytoplasm in the mother cell undergo changes similar to those of the male plant, and after a rearrangement of the plastids a female gamete is formed by the transformation of the whole protoplast. The female gamete, thus formed and containing 24 chromosomes, is discharged from the mother cell.

FERTILIZATION AND GERMINATION

As has been stated, the nuclei in both male and female gametes contain 24 chromosomes. When the female gamete loses its motility and becomes quiescent, a free swimming male gamete becomes attached to it and the union of the two protoplasts occurs. For the sake of brevity, the details of the fusion of the two nuclei, following the union of the gametes, will be omitted in this note.

The fusion nucleus in the common mass of male and female cytoplasm rests for a certain length of time. The first segmentation division takes place within twenty-four hours or less after the union of the gametes. So long as the fusion nucleus remains in the resting state, the round contour of the sporeling is still kept, but when the nucleus has begun to show the early prophase, there is noticed at once at a certain part of the sporeling a slight protuberance, and the cell wall of the protuberance is seen to be considerably thickened. The axis of the mitotic figure of the first segmentation is always parallel with the elongated direction.

The number of chromosomes appearing in the prophase is 48. When these chromosomes become arranged in the equatorial plate, the intranuclear figure is well marked between the kinoplasmic masses

at the poles. After the organization of daughter nuclei the central spindle disappears, and the formation of a cell plate at the expense of the cytoplasm begins only after the nuclei have grown to a considerable size. During the second and ensuing divisions the same number of the chromosomes is present.

Thus the sporeling from the fertilized gametes of *Culleria multifida* develops into a structure of the Aglaozonia form of this species, which contains 48 chromosomes.

Aglaozonia reptans

ZOOSPOROGENESIS

The forms which evidently fall under the category of this species show somewhat wide variability in their habit. The mitosis in the vegetative cells of the form was studied. Since the essential features of the division are similar to those of Cutleria, detailed accounts will be omitted at this time. The fundamental difference between the two forms is that the nucleus of Aglaozonia contains 48 chromosomes, the number persisting up to the formation of the zoosporangium.

Zoosporangia are produced on the upper surface of the thallus. The origin of the structure is as follows: A superficial cell of the thallus slightly elongates and divides, giving rise to two cells, the upper one of which becomes as a rule a zoospore mother cell; not infrequently, however, several cell divisions take place, and in that case the terminal cell becomes the mother cell. The growth of the zoospore mother cell is striking; it elongates until its length becomes three to six times its width. The elongation of the cell is always accompanied by the growth of the nucleus, which remains in the middle region of the cell.

When the nucleus is approaching the prophase, the chromatin network, by a possible rearrangement of the material, becomes less and less branched, and finally there results a tangled mass of continuous threads traversing the nuclear cavity. The tangled threads, becoming more and more uniform in thickness, become transformed into regularly arranged loops centering at a certain part of the cavity, which represents the beginning of the synapsis stage. The synaptic accumulation of the threads occurs always in association with the

kinoplasmic mass outside the nucleus, but its relation to the axis of the cell is varied.

The loops shorten and thicken, and finally they break up into 24 bivalent chromosomes, each derived from one of the loops. The heterotypic figure thus established is intranuclear, and the axis of the spindle is in various directions. Between the first and second divisions the daughter nuclei rest. The four nuclei which are products of the second division contain 24 chromosomes, and the same number is found in the third division which gives rise to eight nuclei.

When the zoospore mother cell has reached the eight-nucleate stage, there occurs generally a cleavage of the cytoplasm, which divides the whole contents of the mother cell into eight zoospore primordia (*Anlagen*). Not infrequently, however, one or two more divisions occur after the third, and as a consequence there are produced 16 or 32 nuclei, and in those cases 16 or 32 zoospores are formed.

The nuclear divisions in the mother cell, as well as the segmentation of the zoospore primordia, always occur simultaneously. As was stated before, the chromosomes contained in the thallus of Aglaozonia are reduced to one-half during the first two divisions in the zoospore mother cell, and 24 chromosomes are involved in the zoospores.

The zoospore germinates independently, without any conjugation; possibly 24 chromosomes, the reduced number, may persist in the structure arising from the germination of the sporelings of the zoospores, but the nuclear details in the sporelings have not yet been completely investigated.

Summary

The nuclear conditions during the life-history of *Cutleria multifida* and *Aglaozonia reptans* may be summarized as follows:

1. The nucleus of both male and female plants of *Cutleria multifida* contains 24 chromosomes; and the male and female gametes produced contain the same number.

2. In the union of gametes the number is doubled, and 48 chromosomes appear in the sporelings, which develop into the Aglaozonia form of Cutleria. Therefore it is evident that the individual bearing the name of *Cutleria multifida* represents the gametophytic phase of

the species, 24 being the gametophytic number of chromosomes; and the Aglaozonia form of Cutleria represents the sporophytic phase of the species, 48 being the sporophytic number.

3. *Aglaozonia reptans* contains 48 chromosomes, and the number is reduced in zoospore formation, the zoospore containing 24 chromosomes. The zoospore with the reduced number of chromosomes germinates without conjugation. Although the nuclear details of the sporelings of *Aglaozonia reptans* have not yet been followed, it seems evident that *Aglaozonia reptans* represents the sporophytic phase of the individual whose gametophytic and sporophytic numbers of chromosomes are respectively 24 and 48. Probably *Aglaozonia reptans* as it occurs in nature is identical with the Aglaozonia form of *Cutleria multifida* which we have grown under culture and is now determined to be the sporophytic phase of the species.

THE UNIVERSITY OF CHICAGO

BRIEFER ARTICLES

OXYGEN PRESSURE AND THE GERMINATION OF XANTHIUM SEEDS

A PRELIMINARY REPORT

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 130

Progress in the analysis of the conditions which cause delayed germination in seeds has been disappointingly slow, largely due to the fact that investigators have persisted in neglecting the seed coat as a factor on account of its thinness, as in KINZEL'S recent paper,[1] or because of a preconceived idea that the embryonic protoplasm is more or less dormant in newly ripened seeds, as in FISCHER'S work,[2] and that some stimulus is necessary to activate it.

It has been shown by CROCKER[3] that the seed coat of Xanthium, in spite of its thinness, is the sole cause of delay under normal germinative conditions. He has also shown[4] that the delayed germination in seeds of aquatic plants depends in many instances on the seed coats. It is evident that the testa cannot be neglected as a factor until its insignificance in that rôle has been proven. If seeds are tested with coats removed, it is possible that no dormant protoplasm will be found needing ionic or other stimulus to growth. Investigations in which the seed coat has been arbitrarily neglected for any reason will have to be repeated before a satisfactory interpretation can be attempted.

On the other hand, it is certain that there are cases in which the delay must be attributed to protoplasmic characters, as in Crataegus, the testa of whose seeds has been proven not to be the cause of the delay in germination. The conditions for protoplasmic activity in the embryo of plants have not been analyzed carefully. A series of experiments is being conducted with the seeds of *Xanthium pennsylvanicum* to determine the exact

[1] KINZEL, WILHELM, Lichtkeimung. Einige bestätigende und ergänzende Bemerkungen zu den vorläufigen Mittheilungen von 1907 und 1908. Ber. Deutsch. Bot. Gesells. **26a**:631–645. 1908.

[2] FISCHER, ALFRED, Wasserstoff und Hydroxylionen als Keimungsreize. Ber. Deutsch. Bot. Gesells. **25**:108–122. 1907.

[3] CROCKER, WILLIAM, Rôle of seed coats in delayed germination. BOT. GAZETTE **42**:265–291. 1906.

[4] ———, Germination of seeds of water plants. BOT. GAZETTE **44**:375–380. 1907.

amount of oxgyen necessary for germination with the coats removed. TAKAHASHI[5] has shown that rice germinates in total absence of free oxygen, and CROCKER[6] has shown the same to be true for *Alisma Plantago* and Eichhornia. But the seeds of Xanthium with coats removed remain dormant if oxygen is entirely excluded, though all other germinative conditions are supplied.

The oxygen pressure necessary for the germination of seeds of *X. pennsylvanicum* has been determined with apparatus similar to that used by SCHAIBLE,[7] with modifications to exclude light and to control the temperature. The seeds are soaked in ice water for twelve hours, and the coats carefully removed, thus excluding them as a factor. The temperature was uncontrolled during the first experiments, but it was found immediately that high temperatures would yield results differing from those at low temperatures. The jars, therefore, were kept in a water bath with cold water running through it constantly. The variation in temperature was not more than about two degrees during the time of each experiment. The seeds used at pressures of less than 99^{mm} of mercury were collected in the spring, after lying in the field nearly six months. Those at 99^{mm} and above were collected in the fall as soon as ripe, and were kept in an unheated dry room during the winter and succeeding spring. Each lot of seeds was put on wet absorbent cotton and was subjected to certain conditions of pressure for ten days. The elongation of the hypocotyl, followed by the geotropic response, was used as a criterion of germination.

Since the desired oxygen pressure is secured by a reduction of total atmospheric pressure, the question naturally arises whether the reduction of pressure itself has any influence on the germination. Experiments are being conducted using the same oxygen pressures at full atmospheric pressure to determine whether the mere difference in pressure is a factor. As some time must elapse before these can be continued, I present the results of the first series of experiments in the accompanying table.

The effect of high temperature is seen by comparing the two experiments at 72^{mm}. The experiment at 99^{mm} was conducted with seeds that had been kept in the laboratory over winter, and the temperature averaged nearly 2° lower than the one at 90^{mm}, so that the percentage of germination was slightly less than at 90^{mm}, in spite of the increased oxygen pressure.

[5] TAKAHASHI, T., Is germination possible in absence of air? Bull. Coll. Agr. Tokyo **6**:439–442. 1905.

[6] CROCKER, WILLIAM, Longevity of seeds. BOT. GAZETTE **47**:69–72. 1909.

[7] SCHAIBLE, FR., Physiologische Experimente über das Wachstum und die Keimung einiger Pflanzen unter vermindertem Luftdruck. Beiträge Wiss. Bot. **4**:93–148. 1900.

It is perfectly clear from the figures given that the oxygen pressure necessary for germination is quite low, and that the pressure is not the same for the two seeds. The uppers require a higher pressure than the lowers; this is a real physiologic difference between the two seeds. It must be noticed that the difference in the embryo in the two seeds is in the same direction as the difference in their seed coats, both sets of characters acting in conjunction, not in opposition, in causing a longer delay in the uppers than in the lowers. However, the difference is so slight in the embryonic characters that the germination of the uppers is not at all hindered if the seed coats are off, with full atmospheric pressure. The uppers begin to germinate on the average just a few hours later than the lowers under such conditions.

Atmospheric Pressure	Oxygen Pressure	Temperature	Percentage germ. in 10 days				Growth in length of hypocotyl in 10 days (mm)			
			Lowers	Control	Uppers	Control	Lowers	Control	Uppers	Control
99mm	20.72mm	19–22°	75	100	45	95	14.5	30.0	4.9	23.3
90	18.84	21–22.6	80	95	50	100	22.8	45.9	4.3	37.8
*72	15.07	20–28	45	100	20	100	11.5	46.0	9.4	33.6
72	15.07	20–22	30	95	0	100	6.36	28.5	0.0	22.0
*28	5.86	21.5–24.5	0	100	0	95	00.0	37.8	0.0	28.8

*Temperature not controlled.

The seeds which failed to germinate under the experimental conditions of pressure and moisture were in every case brought into normal germinating conditions at the close of each experiment. Germination of 100 per cent. in nearly every instance shows that the seeds are not injured by the experimental conditions.

The surprising feature of the results is the small amount of oxygen pressure necessary for germination. From the rapid exchange of gases which Crocker has shown takes place in the seeds of the cocklebur, one would expect to find a rather high pressure required. The results I have obtained are inconsistent with the rapid respiration which he has shown to occur.

Two things must be taken into consideration in regard to this apparent contradiction of results. In the first place, the seed coats are probably responsible for a large amount of the respiration observed in the seeds of Xanthium with the coats intact. Becquerel[8] has shown that the integuments of seeds produce CO^2 quite freely, often showing a larger output than the seeds from which they are taken.

[8] Becquerel, Paul, Recherches sur la vie latente de graines. Ann. Sci. Nat. Bot. IX. 5: 193–320. 1907.

Moreover, there is a strong correlation between the growth of the hypocotyl and cotyledons. Normally the former grows first, and the latter do not enlarge until the root is well established. But if the seeds with coats on are placed in an atmosphere composed largely of oxygen, this normal correlation is reversed, the cotyledons elongating before the hypocotyl begins to develop. The testa is comparatively thick over the hypocotyl, very thin over the cotyledons, and certainly admits oxygen more quickly to the cotyledons than to the hypocotyl. The cotyledons are less sensitive than the other parts of the embryo, and require more oxygen to activate them than would be necessary for the hypocotyl. It is perfectly clear, then, that much of the oxygen used by seeds which germinate with the seed coats intact and in high oxgyen pressure is due to consumption of oxygen by the seed coats and the cotyledons, very little being used by the hypocotyls. In my experiments the pressure has been determined for the very sensitive hypocotyl, which always grows first if the coat is off, and the pressure required is low. I believe that these two points fully explain the difference in oxygen pressure necessary to germination with coats off and coats intact.

Further work is necessary to determine the exact relation of temperature to the oxygen pressures required, and series at high temperatures will be compared with series at low temperatures to obtain definite data on this point.

Fresh seeds will be collected this fall and tested immediately after they have ripened, to determine whether there is any after-ripening, whether the oxygen pressure necessary for germination is greater or smaller before the period of drying, freezing, and resting than it is later.

Acknowledgments are due to Dr. WILLIAM CROCKER, under whose direction the work here recorded was done.—CHAS. A. SHULL, *Transylvania University, Lexington, Ky.*

CURRENT LITERATURE

BOOK REVIEWS

An American memorial to Darwin

The American Association for the Advancement of Science organized at its Baltimore meeting last Christmas week a worthy celebration of the centennial of DARWIN's birth and the semicentennial of the publication of the *Origin of species*, part of which consisted of a series of ten addresses by prominent biologists, on topics pertinent to the occasion. These addresses have been published as a memorial volume, under the title *Fifty years of Darwinism*.[1] Of the ten only two are by botanists: "The theory of natural selection from the standpoint of botany," by JOHN M. COULTER, and "The direct influence of environment," by D. T. MACDOUGAL. Yet all will have a definite interest for any botanist who is alive to the questions of evolution.

Professor COULTER, after pointing out the indebtedness of botany to DARWIN for much besides the theory of natural selection, avows that he speaks not for botanists as a whole, but as one "who has had some experience in dealing with facts that enter into phylogenies." This leads him to set forth some of the difficulties encountered, such as the origination of those broad characters that distinguish great groups, and the so-called "adaptations" which prove to be useless or even harmful. The main illustrations are drawn from the gymnosperms, of which group he is a master; and the array is certainly formidable.

Dr. MACDOUGAL discusses the reaction of organisms when subjected to changes in the environment, and the mechanism by which the structural and formal alterations are effected. He cites the recent experiments of RIDDLE, GAGE, TOWER, GAGER, and himself, all of which are well known and have awakened the greatest interest. He believes that GAGER's experiments and his own indicate that the primary effect is wrought upon the chromosomes of the germ cells; a conclusion that finds support also from GATES's work on Oenothera and from animal cytology.—C. R. B.

The distinctly zoological addresses are: "Isolation as a factor in organic evolution," by DAVID STARR JORDAN; "The cell in relation to heredity and evolution," by E. B. WILSON; "The behavior of unit characters in heredity," by W. E. CASTLE; "Mutation," by C. B. DAVENPORT; "Adaptation," by CARL

[1] Fifty years of Darwinism: modern aspects of evolution. Centennial addresses in honor of CHARLES DARWIN before the American Association for the Advancement of Science, Baltimore, Friday, January 1, 1909. 8vo. pp. vi+274. *pls.* 5. *fig.* 1. New York: Henry Holt & Co. 1909. $2.00.

H. EIGENMANN; "Darwin and paleontology," by H. F. OSBORN; and "Evolution and psychology," by G. STANLEY HALL.

These addresses, by men whose work and writings are concerned with various phases of animal life, deal with problems of wide interest, but in the main they are from the point of view of the zoologist rather than of the botanist, and the material for illustration is largely drawn from animal biology. One gets the impression in reading the different essays that often the point of view of the authors is all too narrow; it is that of the advocate or pleader seeking undue prominence for a certain phase of the evolution problem, rather than that of a man of science considering the phenomena from a broad, impartial point of view.

On the whole, however, the papers are clear, concise, and very much to the point. Due allowance being made for the standpoint from which the individual author took his departure, they present very fairly the average opinions held by the various workers in different fields today. Probably the lay reader will get from the volume what every biologist knows to be true, that the problems of evolution are no longer in the simple state where one factor can be held to explain everything, but that many more factors must be brought in and all harmonized before the evolution question will assume a more unified aspect. He can hardly fail to see clearly that the fact of evolution is beyond dispute, and that it is the method of evolution which is now under discussion.—W. L. TOWER.

Report of American Breeders' Association

The Fifth Annual Report of the American Breeders' Association[2] maintains the high standard which has been shown by all the previous reports of this important organization, and without question contains the most important body of experience and speculation dealing with matters of heredity and breeding as well as of the new science of "eugenics" to be found in America. The present volume is in several respects an improvement over any previous one. A number of useful summaries of breeding work in different crops are given in the form of committee reports, the most important of these dealing with alfalfa, apples, wheat, sugar cane, and tobacco. Special methods for the conduct of practical breeding work in corn, alfalfa, wheat, and sorghum are given. Of over eighty separate articles contained in the volume nearly half have to do with plant breeding, about one-third with animal breeding, and smaller numbers with eugenics, the theory of heredity, and other allied subjects. The most important papers, from a theoretical point of view, are "Some observations in telegony" by E. H. RILEY; "Another mode of species forming," by LUTHER BURBANK; "Some cytological aspects of cotton breeding," by LAWRENCE BALLS; "Characteristics of Wealthy apple seedlings," by W. T. MACOUN; "Clonal or bud-variation," by HERBERT J. WEBBER; "What are 'factors' in Mendelian explanations," by T. H. MORGAN; "Factors for mottling in beans," by R. A. EMERSON; and "The effect of different methods of selection on the fixation of hydrids," by W. J. SPILLMAN. There are

[2] American Breeders' Association. Vol. 5. pp. 420. July 1909. Washington, D. C.

also excellent discussions of work in the production of disease-resistant varieties of flax and wheat by H. L. BOLLEY, and of rice by CHARLES E. CHAMBLISS.

The volume is illustrated with about seventy cuts and diagrams, most of the former being good half-tone engravings. The book is printed on rather cheap paper, but is well bound. It is marred by an undue number of typographical errors, owing to the unfortunate fact that the various papers were not submitted to their authors for correction. The volume closes with a directory of the members of the association, now numbering something over 1200, followed by subject and author indexes to the articles contained in the volume. No biological library can afford to be without these annual volumes, and every one interested in any subject related to heredity or breeding should not fail to become a member of the organization.—GEO. H. SHULL.

Floral biology

Under the general direction of Dr. TOULOUSE, Doin & Fils, Paris, have undertaken the publication of an *Encyclopédie scientifique*. It is divided into 40 sections, each in charge of a special director, and the completed work will comprise about 1000 volumes, each one of which will be a scientific monograph. The classification is exceedingly interesting, botany being represented by three of the 40 sections as follows: 15, *Physiologie et pathologie végétales;* 22, *Botanique;* 35, *Botanique appliquée et agriculture;* not to mention other sections entitled *Biologie, Physiologie, Pathologie*, etc., which inferentially contain no botany. The section of plant physiology and pathology is under the special direction of L. MANGIN, and is to include 13 monographs, the first one of which to appear is on "Floral biology," by PÉCHOUTRE.[3]

The boundaries of the subject are vague, but after a historical introduction the author presents his material in two parts: (1) Sex and sexual elements, and (2) Pollination and floral structures. The topics of the second part are obvious, and the usual information concerning cleistogamy, dichogamy, etc., is presented as fully as 175 pages will permit, and presumably in a form suited to the prospective audience. Just what may be treated in such a volume under the head of "sex and sexual elements," however, is not so self-evident. In this case the titles of the six chapters are in substance as follows: the separation of sexes in flowering plants; the influence of external agents on the determination of sex in dioecious plants; the phylogeny of the separation of sexes; the transformation of "hermaphrodite" plants into dioecious plants, including "slow variation or mutation;" the sexual elements of "phanerogams," including protection of pollen, formation of gametes, the development of the pollen tube, and fertilization; the dissociation of the vegetative and sexual activities of pollen.

Taking the book as a whole, it is conspicuous for its lack of perspective, perhaps it would be better to say its curious perspective; for its material of very

3 PÉCHOUTRE, F., Biologie florale. 12 mo. pp. 369. *figs. 82.* Paris: Octave Doin & Fils. 1909. 5*fr.*

unequal values, assorted in such a way as to indicate information that comes from reading rather than knowledge that comes from investigation; and for its evidence of a very limited acquaintance with the modern literature, including as it does some of the best literature, dealing with the subjects presented. The desire to interpret science to the reading public is a worthy motive, and it ought to appeal more strongly to men of science than it does; but the interpretation must represent current science, or it will deceive rather than inform.—J. M. C.

MINOR NOTICES

Das Pflanzenreich.[4]—Part 38 of this work consists of a monographic treatment of the Cyperaceae-Caricoideae by the distinguished caricologist Professor GEORG KÜKENTHAL. The author follows the usual sequence of this excellent series of monographs in the general consideration of the group. Four genera are included, namely, Schoenoxiphium (6 species), Cobresia (29 species), Uncinia (24 species), and Carex (793 species). The total number of species representing the four genera, as here treated, includes only about a dozen which are characterized as new, and of the new species not one is recorded from America. Several new American varieties, however, are described. The nomenclatorial changes are relatively few. The chief interest of the publication centers on the genus Carex, which is divided into four subgenera and fourteen sections; the divisions are based primarily on the characters of the inflorescence. The keys preceding the species of each section are concise and well contrasted, the descriptions are carefully drawn, the literature and exsiccatae are freely cited, and the illustrations are numerous and well selected. On the whole the present monograph should materially aid toward a better understanding of this difficult but interesting genus.

Part 39 contains an elaboration of the Phytolaccaceae by DR. HANS WALTER. The author recognizes for this family 24 genera and 114 species, of which 32 are new to science. In addition to the general index there is a list of the collectors mentioned and numbers cited in the body of the work, which facilitates greatly the organizing of herbarium material in accordance with the text.—J. M. GREENMAN.

Chronology of the flora of Italy.[5]—The present volume is an analysis of the flora of Italy with particular reference to its historical development. The main body of the work is essentially a catalogue of the species, including those indigenous, introduced, and naturalized, also those in cultivation. The sequence of the genera is in accordance with ENGLER and PRANTL'S *Natürlichen Pflanzenfamilien*. About 4100 species, many varieties, and hybrids are listed, and under the species reference

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[4] ENGLER, A., Das Pflanzenreich. Heft 38 (IV. 20). Cyperaceae-Caricoideae von GEORG KÜKENTHAL, pp. 824. *figs. 128 (981)*. *M* 41.20. Heft 39 (IV. 83). Phytolaccaceae von HANS WALTER, pp. 154. *figs. 42 (286)*. *M* 7.80. Leipzig: Wilhelm Engelmann. 1909.

[5] SACCARDO, P. A., Chronologia della flora Italiana. Royal 8vo. pp. xxxvii + 390. Padova: Tipografia del Seminario. 1909. L.15 ($2.90).

is made to the first record of the plant in Italian literature, as well as subsequent mention by later writers. This volume is the result of much painstaking labor, and it presents a mass of historical information in epitomized form. An excellent bibliographical catalogue adds materially to the value of the publication as a work of reference.—J. M. GREENMAN.

NOTES FOR STUDENTS

Current taxonomic literature.—H. DE BOISSIEU (Bull. Soc. Bot. Fr. IV. 9:348–355. 1909) describes several new species and varieties of Umbelliferae from China, and proposes a new genus (*Chaerophyllopsis*) of this family, which is referred to the tribe Ammineae.—J. D. HOOKER (Kew Bull. 1909: 281–280) in a "Review of the known Philippine Islands species of Impatiens" recognizes 25 species and precedes their enumeration by a determinative key.—C. H. WRIGHT (*ibid.* 308) has published a new genus (*Neodregea*) of the Liliaceae from South Africa.—F. J. SEAVER (Mycologia 1:177–207. *pl. 13.* 1909) under the title "The Hypocreales of North America II" gives a systematic treatment of the tribe Creonectrieae, recognizing 11 genera to which are definitely referred 38 species; 11 additional species are mentioned as of doubtful generic affinity. Five of the genera (*Sphaerodermatella, Creonectria, Macbridella, Scoleconectria,* and *Thyronectroidea*) are new, and of the 38 species 29 form new combinations.—F. D. KERN (*ibid.* 208–210) records a new species of Gymnosporangium from Colorado.—F. D. HEALD (*ibid.* 215–217. *pl. 14*) describes and illustrates a new species of Discosia parasitic on pine seedlings at Halsey, Nebraska.—F. OSTERMEYER (Ann. K. K. Naturhist. Hofmus. 22:128–142. 1907–1908) publishes a list of about 300 plants collected in Brazil in 1860 by Dr. THEO. PECKHOLT; the list contains, among other novelties, a new species of Cryptocarya.—F. KRÄNZLIN (*ibid.* 1911–1996. *pls. 3, 4*) under the title "Beiträge zur Kentniss der Gattung Calceolaria" has published new species of this genus from Central and South America.—A. BRAND (Philip. Jour. Sci. 4:107–110. 1909) has described 5 new species of Symplocos from the Philippine Islands.—E. B. COPELAND (*ibid.* 111–115) in continuation of his studies on Philippine ferns records 7 new species and proposes one new genus (*Currania*).—E. D. MERRILL (*ibid.* 117–128) presents a "Revision of the Philippine Connaraceae," recognizing 5 genera and 17 species of which 8 are described as new; the same author (*ibid.* 129–153) under the title "A revision of Philippine Loranthaceae" recognizes 6 genera and 53 species of which 19 are new; one new genus (*Cleistoloranthus*) is proposed.—H. N. RIDLEY (*ibid.* 155–199) gives a synopsis of the Scitamineae of the Philippine Islands. The group includes four families, as follows: Zingiberaceae with 15 genera and 61 species, Marantaceae with 4 genera and 7 species, Cannaceae with 1 genus and 2 species, and Musaceae with 1 genus represented by 1 endemic and 4 cultivated species. Several species are here described for the first time.—A. DECANDOLLE (Leafl. Phil. Bot. 2:633–638. 1909) gives a "Revision of the Philippine species of Elaeocarpus," in which 16 species are recognized, 3 being new.—A. ENGLER (Bot. Jahrb. 43:303–381. 1909), in cooperation with several botanists, under the title

"Beiträge zur Flora von Afrika XXXV" has published 125 new species and several varieties of flowering plants. The following new genera are proposed: *Lingelsheimia*, *Baccaureopsis*, and *Milbraedia* of the Euphorbiaceae, *Pierrina* of the Scytopetalaceae, and *Ledermanniella* of the Podostemonaceae. The contribution is based chiefly on the collections of Dr. J. MILBRAED.—O. MÜLLER (*ibid.* Beibl. No. 100. pp. 1–40. *pls. 1, 2*) lists a large number of Bacillariaceae from southern Patagonia from the collections of E. NORDENSKIÖLD and O. BORGE. Several new species and varieties are described.—E. L. GREENE (Rep. Nov. Sp. **7**: 195–197. 1909) under the title "Novitates Boreali-Americanae IV" has published 7 new species of sympetalous plants.—J. R. DRUMMOND (Curtis' Bot. Mag. IV. **5**: *t. 8271.* 1909) describes and illustrates a new species of Agave from Central America. M. GURKE (Monats. Kakteenk. **19**: 116–121. 1909) describes and figures a new species of cactus (*Cephalocereus DeLaetii*) indigenous to Mexico.—W. FAWCETT and A. B. RENDLE (Jour. Bot. **47**: 263–266. 1909) in continuation of their studies on Jamaican orchids have published 6 new species and include 1 new genus (*Harrisella*) which is based on *Aeranthus porrectus* Reichb.—A. and E. S. GEPP (*ibid.* 268, 269) have described a new species of Udotea from St. Thomas.—W. A. MURRILL (Mycologia **1**: 140–160. 1909) in a second article on the "Boletaceae of North America" gives a synopsis of the genus Ceriomyces, recognizing 35 species, and (*ibid.* 218, 219) describes a new species of this genus from the volcano of Turrialba, Costa Rica.—E. ROSENSTOCK (Rep. Nov. Sp. **7**: 146–150. 1909) under the title "Filices Novae V" describes new species of ferns, 3 of which are from Ecuador.—F. C. CLEMENTS and H. L. SHANTZ (Minn. Bot. Studies **4**: 133–135. *pl. 20.* 1909) have proposed a new genus (*Eucapsis*) of the blue green algae; the genus is represented at present by a single known species (*E. alpina*) from Colorado.—C. H. PECK (Bull. Torr. Bot. Club **36**: 153–157. 1909) has published 10 new species of North American fungi.—J. K. SMALL (*ibid.* 159–164) in an article entitled "Additions to the flora of peninsular Florida" records several species hitherto unknown from the mainland and describes 5 new species.—A. D. E. ELMER (Leafl. Philip. Bot. **2**: 595–629. 1909) has described 11 species and 2 varieties of Philippine plants as new to science. Synopses of the Philippine species of Fagraea, Artocarpus, and Hydrocotyle are given, and a new generic name (*Adelmeria* Ridl.) is proposed to take the place of Elmeria recently described in this journal.—J. M. GREENMAN.

Corn breeding.—Several recent papers have appeared advocating the use of hybridization methods in the production of Indian corn, instead of the usual ear-to-the-row method which is based upon the idea of isolation of pure types. As early as 1893 and 1894 GARDNER and MORROW[6] showed that crosses between different strains of corn give somewhat increased yields over either of the parent strains, and a method was outlined by which this advantageous circumstance could

[6] MORROW, G. E., AND GARDNER, F. D., Bulletins 25 and 31, Illinois Agricultural Experiment Station. 1893, 1894.

be readily utilized. Two years ago the reviewer read a paper[7] before the American Breeders' Association in which it was shown that a field of Indian corn consists of a large number of elementary species thoroughly hybridized in complex fashion, and gave evidence that the vigor necessary to the production of large yields is due to the degree of heterozygosis possessed by the individuals composing the crop. This paper closed with the suggestion that "continuous hybridization instead of the isolation of pure strains is perhaps the proper aim of the corn breeder." Based upon this conception the reviewer[8] worked out a scheme of corn breeding in which definite pure lines were isolated and recombined, so that the field crop would consist of first-generation hybrids between these two pure lines, thus insuring perfect uniformity as well as high yield. Simultaneously with this latter paper there appeared two other papers presenting suggestions for a similar method of corn breeding. In the first of these EAST[9] suggests the purchase by the farmer of two highly bred strains from the professional corn breeder, and the hybridization of these two strains each year to produce the seed corn for the field crop, arguing that the methods used by the professional breeder are such as to render these strains already to a considerable extent homozygous. This method is the same as that of MORROW and GARDNER. EAST recognizes the relation between this method and the pure-line method of the reviewer, saying that the latter is more correct theoretically, but less practicable than the method he suggests. EAST gives a clear and incisive discussion of the significance of the pure-line idea. COLLINS[10] has issued a bulletin also advocating the use of continuous hybridization in corn breeding. The appearance of three papers simultaneously advocating the same innovation in corn breeding is likely to have great influence on the activity of those engaged in this work. The bulletin by COLLINS is unfortunately quite vague in its language, as might be inferred from the title, "The importance of broad breeding in corn." Comparing the conception of "broad breeding" with the conception involved in the other two papers appearing simultaneously with it, both of which are expressed in terms of definite hybridization, gives a fair indication of the relation between these papers. In keeping with his title, COLLINS says "had it been realized that diversity is as necessary to the life of the species as is chlorophyll to the life of the individual, it would have been evident that one might as well breed to eliminate the green color from the leaves as to suppress this corn variation." He says also that "the appearance of so-called barren stalks in a field of corn may be thought of as an adaptation to avoid self-pollination," and adds that "the elimination of these

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[7] SHULL, G. H., The composition of a field of maize. Amer. Breeders' Assoc. **4**:296–301. 1908.

[8] ———, A pure line method in corn-breeding. Amer. Breeders' Assoc. **5**:51–59. 1909.

[9] EAST, E. M., The distinction between development and heredity in in-breeding. Am. Nat. **43**:173–181. 1909.

[10] COLLINS, G. N., The importance of broad breeding in corn. U. S. Bureau Plant Industry, Bull. 141[4]:33, 34. 1909.

proterandrous plants results in increasing the percentage of self-pollinated plants, and is a practice of doubtful value."

SMITH[11] has issued a bulletin dealing primarily with the results of selection of ears which are placed high on the stalks and those which are placed low on the stalks, and showing that very material difference may be secured by five years' selection. A comparison is then made between the two strains so produced in regard to qualities such as time of maturity, yield, etc. The results accord very well with the notion that ordinary varieties of corn are much hybridized, and that the selection results in a partial separation of the biotypes involved.—GEO. H. SHULL.

Morphology and sexuality of Aspergillus and Ascophanus.—In *Aspergillus repens*, a form differing slightly in structure from *Aspergillus herbariorum* as described by Miss FRASER and Miss CHAMBERS,[12] Miss DALE[13] describes another case of so-called reduced fertilization among the Ascomycetes. After a brief historical and systematic consideration of the species, she describes the multinucleate hyphae of the mycelium, from which arise the multinucleate conidia as apical swellings. The archicarp is initiated as a slender branch, which usually soon becomes regularly and closely coiled into a spiral. The regular occurrence of definite ascogonia and antheridia as figured by DEBARY was rarely found, the antheridium often being absent. No convincing proof of a fusion of sexual organs, even when both were present, was discovered. Transverse walls, whose position and number vary considerably, appear in the archicarp either very early or at a much later stage. Ascogenous hyphae develop in some cases from all of the cells of the ascogonium. The investing hyphae show great variations in the time at which they arise, as well-developed ascogonia with ascogenous hyphae quite uninvested are often found. The young archicarp, which arises as a multinucleate branch, possesses nuclei of about uniform size. Later variations in size of the nuclei appear, which Miss DALE accounts for chiefly by a fusion in pairs, although nuclei may perhaps grow. Such nuclear fusions are figured in all cells of the ascogonium. Since no antheridium is believed to fuse with the oogonium, these nuclear fusions are held to be reduced sexual ones. These fusion nuclei pass into the ascogenous hyphae. In the development of the ascus, which arises from the penultimate cell of a hypha, the usual nuclear fusions and subsequent triple divisions occur. Karyokinesis was not observed.

CUTTING[14] finds in *Ascophanus carneus* still another case of reduced fertiliza-

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[11] SMITH, L. H., The effect of selection upon certain physical characters in the corn plant. Ill. Agric. Exper. Sta. Bull. 132:50–60. 1909.

[12] FRASER, Miss H. C. I., AND CHAMBERS, Miss H. S., The morphology of *Aspergillus herbariorum*. Ann. Mycol. **5**:419–431. 1907.

[13] DALE, Miss E., On the morphology and cytology of *Aspergillus repens* DeBary. Ann. Mycol. **7**:215–225. 1909.

[14] CUTTING, E. M., On the sexuality and development of the ascocarp of *Ascophanus carneus* Pers. Annals of Botany **23**:399–417. 1909.

tion. The fungus, whose spores start to germinate in alkaline media, was not successfully cultivated in the laboratory. The cross-walls of the multinucleate cells of the vegetative hyphae have a pore, on each side of which are a number of deeply staining granules, whose function was not determined. The archicarp, a scolecite arising as a branch from a vegetative hypha, consists of a basal vegetative portion, a central ascogonial part, and a terminal vegetative region, which is regarded as a functionless trichogyne. The number of cells in each of these regions varies greatly. From the basal region numerous investing hyphae arise. Although the ascogonia grow crowded together, each fruit arises from a separate ascogonium. The cells of the ascogonial portion have each a pore in their transverse walls, which is guarded by small granules early fusing together to form a pad closing the pore. This pad eventually disappears, leaving the multinucleate ascogonial cells in communication. Nuclear fusions are believed to occur in all of the cells of the ascogonial portion. No nuclear migrations were observed, fusions occurring even before the pads disappear. Ascogenous hyphae arise from any or all of the ascogonial cells. Each ascogonial cell is regarded as female, and in the absence of an antheridium the nuclear fusions are held to represent a type of reduced fertilization. The asci develop from the penultimate cells of the recurved tips of the ascogenous hyphae in the usual way. The usual nuclear fusions and divisions occur in the development of the ascus. No karyokinetic figures were observed, and the method of spore-formation is not described.—J. B. OVERTON.

Respiration and fermentation.—KOSTYTSCHEW, in a preliminary paper,[5] points out that "the metabolism of the complex processes of vital oxidation remain yet quite unexplained, chiefly because the oxidases, according to the latest researches, are unable to produce a direct combustion of the carbohydrates." Conceding that anaerobic respiration is identical with alcoholic fermentation, he enumerates the possibilities as to the rôle of zymase in respiration: (1) the zymase of seed plants is not identical with that of yeast; (2) alcoholic fermentation in seed plants occurs in the presence of O_2, but has nothing to do with aerobic respiration; (3) alcoholic fermentation is the first stage of aerobic respiration, the alcohol formed being oxidized to CO_2 and H_2O; (4) alcoholic fermentation is the first stage of aerobic respiration, but in the presence of air under normal conditions no alcohol is formed, because the intermediate products are oxidized; (5) alcoholic fermentation is the first stage of aerobic respiration, but the alcohol is used as constructive material. Of these possibilities he eliminates several, citing various researches which bear on them, and reports his own investigations, which indicate the correctness of the fourth hypothesis above. The small quantities of alcohol that have been observed by some investigators are easily accounted for by the assumption that the oxidative power of the plant does not always keep exact pace

[5] KOSTYTSCHEW, S., Ueber den Zusammenhang der Sauerstoffatmung mit der Alkoholgärung. Ber. Deutsch. Bot. Gesells. **26**: 565–573. 1908.

with the formation of primary cleavage products, in which case these are elaborated into alcohol and CO_2.—C. R. B.

A new fossil araucarian.—SINNOTT[16] has described a new genus of araucarian wood from the Cretaceous (?) clays of Scituate, Mass. The structure of *Paracedroxylon scituatense* consists of tracheids and pith rays, the radial pits of the former being circular (not flattened by mutual contact), and the cells of the latter thin-walled and without pits except in the walls adjacent to the tracheids. Groups of thin-walled cells which occur in wounded regions are thought possibly to represent abortive resin canals, and in the bands of wound tissue which occur near wounds large anastomosing mucilage spaces appear which are said to represent somewhat modified traumatic resin canals. The conclusion is that Paracedroxylon is another primitive araucarian which is "on the border line between this group and their ancestors, the primitive Abietineae," which "probably left the ascending araucarian line before the appearance of flattened pitting," and whose "traumatic canals were subsequently much reduced from the typical abietineous condition."—J. M. C.

Oospheres of Sargassum.—In a short preliminary note, TAHARA[17] announces the periodic liberation of the oospheres of the species of Sargassum (about 10 in number) at the Misaki Marine Biological Station of the Tokyo Imperial University, *S. enerve* being the species chiefly under observation. The liberation occurs simultaneously not only in a given individual, but also in all the plants of the locality, proceeding in fortnightly crops on a particular day, at a fixed interval after the highest spring tide, this interval varying in different species. All the oospheres of a single conceptacle are not discharged at one time, but in two or three successive fortnightly crops.—J. M. C.

Light and protein synthesis.—ZALESKI, after further experiments,[18] has supported the general view that light promotes the synthesis of proteins only indirectly, because of the relation to the synthesis of carbohydrates. He rejects the researches of LAURENT and of GODLEWSKI as insufficient to show the direct influence of light upon any molecular combination in the proteins.—C. R. B.

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[16] SINNOTT, EDMUND A. W., Paracedroxylon, a new type of araucarian wood. Rhodora **11**:165–173. *pls. 80, 81.* 1909.

[17] TAHARA, M., On the periodical liberation of the oospheres in Sargassum. Preliminary note. Bot. Mag. Tokyo **23**:151–153. 1909.

[18] ZALESKI, W., Ueber dei Rolle des Lichtes bei der Eiweissbildung in den Pflanzen. Ber. Deutsch. Bot. Gesells. **27**:56–62. 1909.